(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 April 2003 (17.04.2003)

PCT

(10) International Publication Number
WO 03/031430 A2

(51) International Patent Classification[7]: C07D 311/62, 311/30, C07H 17/065, 17/07, A61K 31/352

(21) International Application Number: PCT/EP02/11181

(22) International Filing Date: 4 October 2002 (04.10.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

RM2001A000600	4 October 2001 (04.10.2001)	IT

(71) Applicant *(for all designated States except US)*: **BRANE TECH S.R.L.** [IT/IT]; Via Dora, 2, I-00198 Roma (IT).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **PORTA, Amalia** [IT/IT]; Via Giardini 1, I-88004 Crotone (IT).

(74) Agent: **GERVASI, Gemma**; Notarbartolo & Gervasi S.p.A., Corso di Porta Vittoria, 9, I-20122 Milan (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

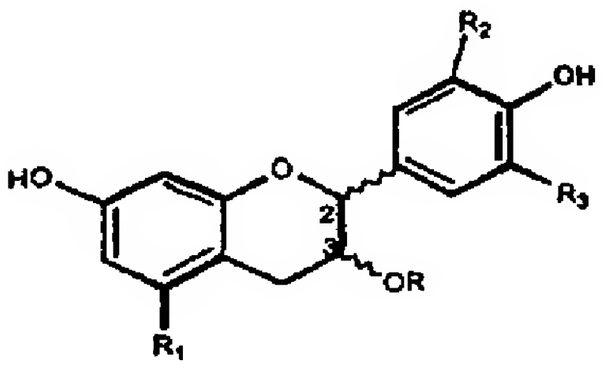
— *of inventorship (Rule 4.17(iv)) for US only*

Published:

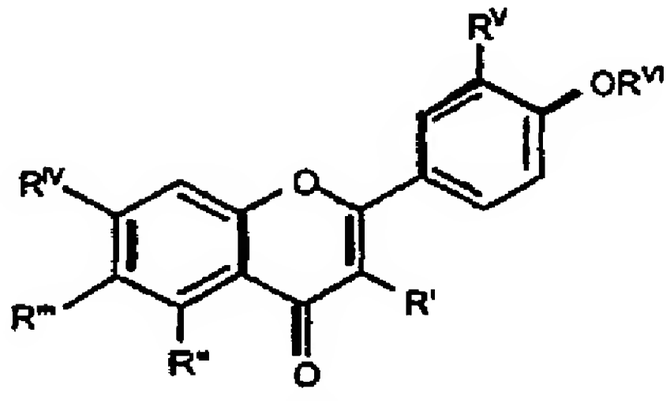
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations. refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FLAVONOID COMPOUNDS CAPABLE OF MODIFYING THE DYNAMIC AND/OR PHYSICAL STATE OF BIOLOGICAL MEMBRANES AND TO STIMULATE THE ENDOGENOUS SYNTHESIS OF STRESS PROTEINS IN EUKARYOTIC CELLS, RELATIVE SYNTHESIS AND THEIR USE



(I)



(II)

(57) Abstract: The invention relates to flavonoids compounds of formula (I) and (II) capable of modifying the dynamic and/or physical state of biological membranes and to stimulate the endogenous synthesis of stress proteins in eukaryotic cells. Such compounds are molecules of plant origin or synthetic. The invention also describes a method to identify, purify and chemically synthesize such flavonoid compounds and test their efficacy through their capacity to stimulate the transcription of stress genes and as a consequence, to interact with biological membranes with alteration of their relative physical state. Such compounds and corresponding pharmaceutically acceptable derivatives and/or salts have applications in the areas of pharmaceuticals, more specifically in cosmetics and dermatology, for all those afections related to an alteration of the expression of stress genes.

WO 03/031430 A2

FLAVONOID COMPOUNDS CAPABLE OF MODIFYING THE DYNAMIC AND/OR PHYSICAL STATE OF BIOLOGICAL MEMBRANES AND TO STIMULATE THE ENDOGENOUS SYNTHESIS OF STRESS PROTEINS IN EUKARYOTIC CELLS, RELATIVE SYNTHESIS AND THEIR USE

Field of the invention

The present invention relates to flavonoid compounds capable of modifying the dynamic and/or physical state of biological membranes and to stimulate the endogenous synthesis of stress proteins in eukaryotic cells, relative synthesis and their use. In particular, such compounds are molecules of plant origin or synthetic. The invention also describes a method to identify, purify and chemically synthesize such flavonoid compounds and test their efficacy through their capacity to stimulate the transcription of stress genes and as a consequence, to interact with biological membranes with alteration of their relative physical state. Such compounds and corresponding pharmaceutically acceptable derivatives and/or salts have applications in the areas of pharmaceuticals, more specifically in cosmetics and dermatology, for all those afections related to an alteration of the expression of stress genes.

Terms used in the following description of the invention

Aglicons The aglicons are those compounds that in the present invention bind sugars residues (e.g. glucose, fucose, xylose, etc.) forming glycosides. If the sugar moiety is made of by one or more molecules of glucose, such compounds are also defined as glucosides. In a glycoside the non-sugary moiety is defined as "agliconic portion". Aglicons and glycosides usually have names recalling the natural source from which they have been isolated for the first time.

Gene expression This term designates a mechanism by which an organism synthesizes a protein coded by a specific gene by accumulating an intermediate mRNA.

Heat shock genes (stress genes): ubiquitarious genes that are rapidly transcriptionally activated when cells are exposed to a sudden increase in temperature and/or to various forms of stresses. Stress inducibility is determined by the presence of specific *cis* elements in the promoter region of this genes (e.g.. heat shock element, HSE).

Gene Reporter are genes whose proteic product is easily measured. They are used to analyze and determine the regulating zones of promoters of specific genes (cis sequences). They are used under the control of a promoter of which the transcriptional activity is to be tested.

L929 cell line Cell line of fibroblasts of murine fibrosarcoma.

MPS: Membrane physical state. In the following description the physical state is intended to comprise also the dynamic state, even when not expressely mentioned.

Membrane: semi-permeable barrier that surrounds eukaryotic and prokaryotic cells, organelles (e.g. mitochondria, chloroplasts, endoplasmic reticulum, nuclei, etc), that is composed by a lipid bilayer in which intrinsic membrane proteins or associated proteins are present, and in some cases, cholesterol, ergosterol or glycolipids. All membrane, at different levels among them, undergo cell specific changes in their physical state as a result of the activity of the molecules of the present invention.

Heat shock proteins (HSPs or stress proteins): the protein product of heat shock genes rapidly accumulated by a cell after exposure to stress and whose functions include: assign the proper folding of nascent polypeptides, targeting of denatured proteins (misfolded), protection of mitochondrial and chloroplasts functions, mRNA maturation, their insertion in membrane to protect MPS, etc.

Integral (or intrinsic) membrane proteins: Any membrane protein that, partially or totally, interacts with the hydrophobic region of the phospholipid bilayer and that can be extracted from membrane only by detergents.

PCR (*polymerase chain reaction*)**:** technique to synthesize *in vitro* large amounts of specific nucleotide sequences by the use of specific oligonucleotide primers complementary to sequences of the target gene using special termostable DNA polymerases.

Promotor: a specific DNA region onto which RNA polymerase initiates mRNA transcription. The promoter includes a site for DNA binding recognition.

Signaling transduction pathways: Conversion of a signal from a physical (e.g. or temperature, osmolarity) chemical (e.g. hormones) form into an other. In cell biology, this term is referred to the sequential process initiated by the interaction of a chemical factor with a membrane or cell receptor or a physical effect on

membrane that cu minates in one or more specific cell response (e.g. gene transcriptional activation of sequences under this control).

Transformation: method to obtain proteins through DNA recombinant techniques that requires the cloning of a gene coding for a given protein and where "cloning" means isolation, purification and sequencing of the gene coding for that protein. Once cloned, the nucleotide sequence can be inserted in an appropriate expression vector and the obtained DNA recombinant molecules can be introduced in a microorganism in which the gene is simultaneously replicated with the host DNA. The gene can eventually be re-isolated with standard techniques of molecular biology.

Cloning vector: DNA molecules that contain the entire genetic information that allows them to replicate when transfected in a host.

Membrane fluidity. A widely used but subjective term that describes the relative diffusional motion of molecules within membranes. Fluidity is used rather than viscosity, because membranes are planar, asymmetric structures, and their properties are not comparable to bulk phases. The term fluidity is meant to convey the impression of lateral diffusion, molecular wobbling and chain flexing, that are found in functional membranes where the lipids are in the fluid-crystalline lamellar phase.

Membrane order. The motional movement of molecules or molecular domains within the membrane. Membrane order can be quantified by estimating the motion of paramagnetic probes and calculating an order parameter from the ESR or NMR spectrum.

Non-lamellar phases. Non-bilayer arrangements of lipids in aqueous media. These can be hexagonal (H_I) or inverted hexagonal (H_{II}) arrangements; H_I phase is seldom found in membranes.

Background of the invention

The Heat Shock Response, or stress response, is one of the better studied homeostatic cell responses, mainly involved in the maintenance of cell functionality in response to diverse environmental stresses and/or in pathologic states (Lindquist. 1986). Such response is mediated by a rapid increase in the transcription of those genes that codify for the stress proteins (Morimoto et al. 1998). It has been abundantly demonstrated that such increase in mRNA

synthesis of stress genes, and the relative intracellular accumulation of HSPs, are associated with the acquisition of thermotolerance, with protection to subsequent exposure to other forms of stresses or in pathological conditions, etc. (Singer & Lindquist 1998; van Eden & Young 1996; Morimoto et al, 1998). It has been demonstrated that the primary sensor(s) of temperature variations, and in general to other forms of stresses, is (are) localized in the membrane (Carratù *et al* 1996; Horvath *et al* 1998, Vigh & Maresca, 1998; Suzuki *et al* 2000, Piper *et al* 2000; Torok et al 2001). Further, recent studies have shown that an abrupt temperature change or exposure to other forms of stress, determine a physical re-organization of lipid and protein membrane components (Slater *et al* 1994), that is followed by a specific gene response aimed to compensate variations in MPS. Thus, a cross-talk between changes in MPS and regulation of gene expression exists, particularly for heat shock genes.

Among the agents responsible of an appropriate MPS we mention desaturases that through their enzymatic activities control the membrane phospholipid composition. Desaturases are enzymes that introduce double bonds in saturated fatty acids (SFA) transforming them into unsaturated fatty acids (UFA). The SFA/UFA ratio is one of the main factors that determines an appropriate MPS in all cells (Cossins, 1994). Recently, it has been demonstrated that the inducible synthesis of stress proteins is controlled by a rapid and local variations of several factors:

- the membrane lipid composition
- membrane lipid/protein interactions
- lipid dynamics (MPS changes) (Vigh et al, 1998).

Thus, the MPS changes in stress conditions re-determines the threshold at which HSPs are normally synthesized.

The aim of this invention is to use in cosmetics and pharmacology the properties of some molecules to accumulate endogenous stress proteins. The cosmetic and therapeutic effects are based on the capacity of such molecules to stimulate such molecules that in turn induce intrinsic cellular homeostatic mechanisms that are altered in specific human and animal pathological conditions as well as in the plants. Further, accumulation of stress proteins, whose capacity to induce cell and tissue protection is well known (Edwards et al, 1999; Latchman 1998; Santoro

2000), confers in a specific manner protection from UV exposure, retards aging, protects from environmental stress (e.g. abrupt increase in temperature, dehydration, etc. (van Eden W et al 1996). Therefore, it has been suggested that these molecules by stimulating HS protein synthesis, particularly in the skin, may be utilized as pharmaceutical drugs and as cosmetics. Stress proteins are also involved in the mechanisms of wound healing, in dermatological diseases such as psoriasis (Edwards et al 1999) .

It also known that accumulation of stress proteins is altered in several human chronic diseases such as diabetes, degenerative diseases, such as in the central nervous system, in cancer, in inflammation, in rheumatoid arthritis, in wound healing, in autoimmune diseases, in heart diseases, during aging, etc. (Hightower *et al,* 2000; Polla 1998; Laplante *et al* 1998; van Eden W et al 1996; Feige *et al* 1996; Maytin 1992). It has also been reported that pre-induction of stress proteins acts in a protective manner in several clinical diseases. For example, brief episodes of ischemia, that induce the preferential accumulation of HSP72, protect myocardium from subsequent otherwise lethal ischemia (Sammut *et al* 2001; Marber *et al* 1995). Furthermore, HSP70 reduces the size of the infarct following ischemia (Okubo *et al* 2001). The over expression of rat *hsp70* gene in transgenic mice increases protection from cardiac and cerebral ischemia (Rajdev *et al* 2000; Plumier *et al* 1996; Plumier *et al* 1995). The main pre-requisite of HSP inducible drugs is that they must be non toxic and lack side effects together with the property to mimic the effects of stressing agents or, in the absence of stress or in limited stress or in altered physiological conditions of cell targets, to lower the threshold of stress condition in such a way that signals that induce cascade effects are initiated and that cause the transcriptional induction of stress genes. Several agents that induce heat shock protein accumulation have been identified. However, so far, the only one reported to be non-toxic is bimoclomol™. We have now identified a family of chemical compounds of plant origin, particularly useful to be used to modify MPS, thus inducing an increase in amount of synthesis of stress proteins.

Summary of the invention

There are objects of the present invention flavonoid compounds of a general formula (I) and (II) that can be used in the pharmaceutical field, particularly in

cosmetics, to modify the cell membrane physical state, particularly by increasing the synthesis of stress proteins.

Further objects of the invention are the pharmaceutical compositions comprising, as active principle, the molecules of the general formula (I) and (II) and relative mixtures.

Further object of the present invention is the chemical synthesis to obtain the molecules of the general formula (I) and (II).

Further object of the present invention is the use of molecules of the general formula (I) and/or (II) to treat pathological conditions derived from an alteration of membrane physical state of eukaryotic cells, of plant cells, of animal cells, particularly, mammalian and human cells, with lack of toxicity and/or side effects.

Further objects of the invention are: a method to modify MPS, method to induce stress response, such as heat shock, a method to induce cell protection in eukaryotic cells (e.g. L929, human keratynocytes, etc.) by treatment of cells, tissue or entire animal or plant with an effective amount of the compounds of the general formula (I) and/or (II) and corresponding pharmaceutically effective derivatives and/or salts, including optically active molecules and relative mixtures. Either L929 cells or keratynocytes are preferentially transfected with luciferase genes whose expression is under the control of a human *hsp70* promoter.

A further object is a method for the prevention and/or treatment of related alterations connected with modification of cell membrane physical state in plants as well as in animal cells, particularly human.

There are also objects of the present invention the flavonoid products obtained by extraction and purification from vegetable material that contain them, utilizable according to the invention, to modify MPS and induce heat shock transcriptional activation.

Other objects will be evident from the detailed description of the invention.

Brief description of the drawings

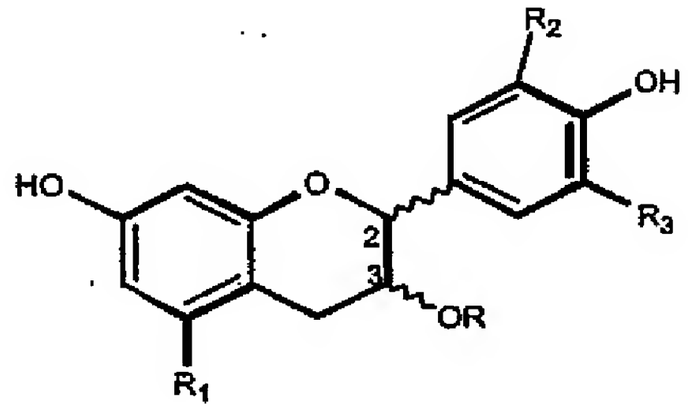
Figure 1. Plasmid vector pGL3 containing luciferase as a reporter gene under the control of the heat shock promoter (HSE element). Further, the vector contains the ampicillin and geneticin resistant genes as selectable markers.

Figure 2. Luciferase assay in L929 cells grown at 37°C, treated with different molecules and in heat shock conditions at 40° and 41°C.

Figure 3. *Test to evaluate changes of MPS in artificial membranes (LUVs, Large Unilamellar Vesicles), made of di-oleil-fosfatidyl-ethanolamine, di-oleil-fosfatidyl-choline, cardiolipin and fosfatidylserine, that mimic biological membrane lipid composition. Fluidity has been measured with DPH (1,6-difenyl-1,3,5-esatriene) measuring fluorescence. In the figure the experimental data of molecules #11 and #100 are reported. Molecule #11 increases fluidity (destabilizes membranes) while #100 rigidifies membranes.*

Detailed description of invention

The present invention is based, at least in part, on the unexpected finding that flavonoid compounds can modify, increasing, the synthesis of stress proteins, as a consequence of the change in MPS that they induce. This finding is significant in the light of the role that HSPs have in the protection of cells from the pathological effects of several diseases. The molecules of the invention are believed to increase stress protein concentration and to protect cells from the side effects of degenerative diseases, such as: tissutal damages, nerve conductivity, membrane cell damage, etc. The molecules of the invention are particularly active in inducing the synthesis of stress proteins such as HSP70, HSP72, HSP90 etc. and small heat shock proteins such as HSP17, HSP20, etc. The compounds according to this invention have the general formula (I) and (II) and both belong to the flavonoid family:



(I)

The compounds represented by the general formula (I) are derivatives of flavonoids in which:

R = H, gallate, glycosidic moiety having a number of sugar residues ranging between 1 and 2 equal or different to each other, preferably selected in the group

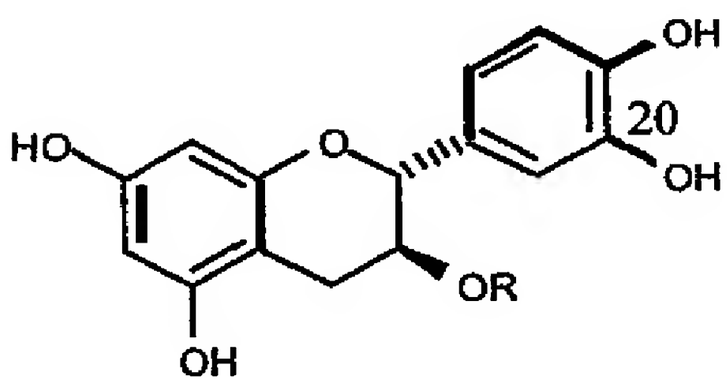
of: β-D-glucose, β-D-mannose, β-D-galactose, β-D-xylose, α-L-arabinose, β-D-quinovose, β-D-fucose, α-L-ramnose, and corresponding mixtures;

R1, R2, R3, equal or different among each other are H or OH.

There are within the scope of this invention the peracetylate derivatives of the compound having formula (I), to say compounds in which OH groups are esterified with acetic acid. They represent important intermediates in the synthesis of the molecules of this invention.

The C atoms in positions (2) and (3) may have configuration *R* and *S*. Molecules of the general formula (I) have two chiral centers, in C 2 and 3, with the possibility to produce 4 different diastereoisomers (different combinations of configurations): [2*R*,3*S*], [2*R*,3*R*], [2*S*,3*R*], [2*S*,3*S*]. Starting from the observation that in nature the configuration *2R* is largely diffused for biogenetic reasons and that glycoside derivatives have a higher biological activity in the reported molecular and biophysical assays, such molecules ere considered more attractive and their synthesis is afterward reported.

Preferred molecules according to the general formula (I) are indicated with the following general formula: derivative of (+)-catechin [2*R*, 3*S*]

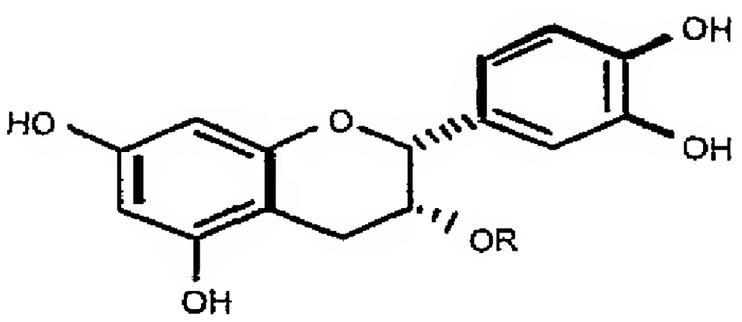


(IA)

in which R is selected in the group of the following substituents:

R=H; R=β-D-glucose*.#(Sasuga et al. 2000, Bae et al. 1994); R=β-D-mannose# (Sasuga et al. 2000); R=β-D-galactose# (Sasuga et al. 2000); R=β-D-xylose# (Sasuga et al. 2000); R=α-L-arabinose; R=β-D-quinovose; R=α-L-ramnose* (Banefeld et al. 1986); R = gallate§

Derivatives of (-)-epicatechin [2*R*, 3*R*]

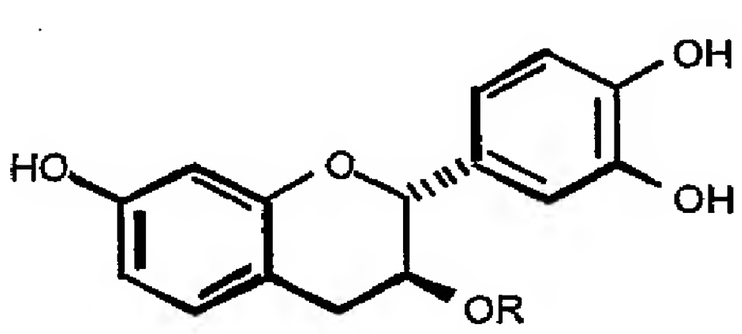


(IB)

in which R is selected in the group of the following substituents:

R=H; R=β-D-glucose* (Morimoto et al. 1986); R=β-D-mannose; R=β-D-galactose; R=β-D-xylose; R=α-L-arabinose; R=β-D-quinovose; R=α-L-ramnose; R=gallate§

Derivatives of (+)-fisetidinol [2*R*, 3*S*]

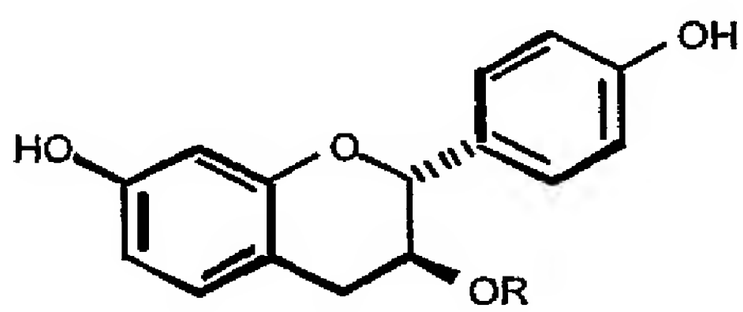


(IC)

in which R is selected in the group of the following substituents:

R=H; R=β-D-glucose; R=β-D-mannose; R=β-D-galactose; R=β-D-xylose* (Piacente et al. 1999); R=α-L-arabinose; R=β-D-quinovose; R=α-L-ramnose; R=gallate

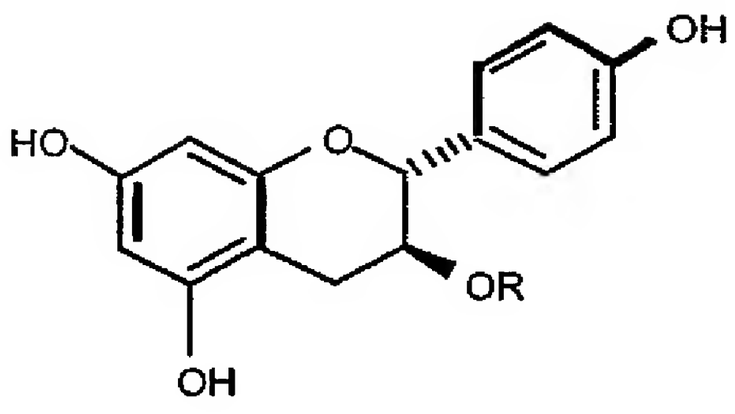
Derivatives of (+)-guibourtinidol [2*R*, 3*S*]



(ID)

in which R is selected in the group of the following substituents: R=H; R=β-D-glucose; R=β-D-xylose; R=β-D-quinovose

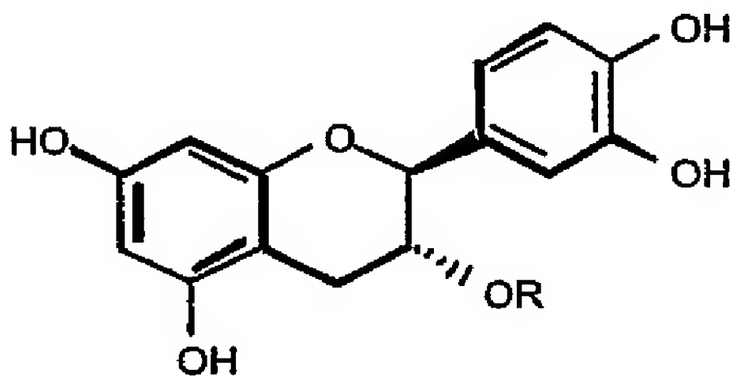
Derivatives of (+)-efzelechin [2*R*, 3*S*]



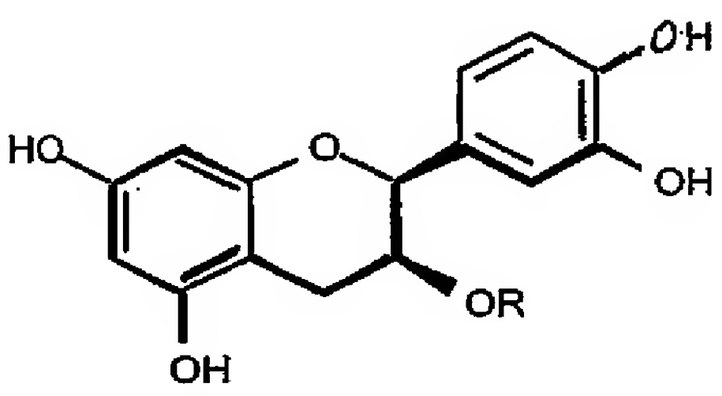
(IE)

in which R is selected in the group of the following substituents: R=H; R=β-D-glucose; R=β-D-xylose; R=β-D-quinovose

Derivatives of flavan-3-olo with 2*S* stereochemistry



(IFa)

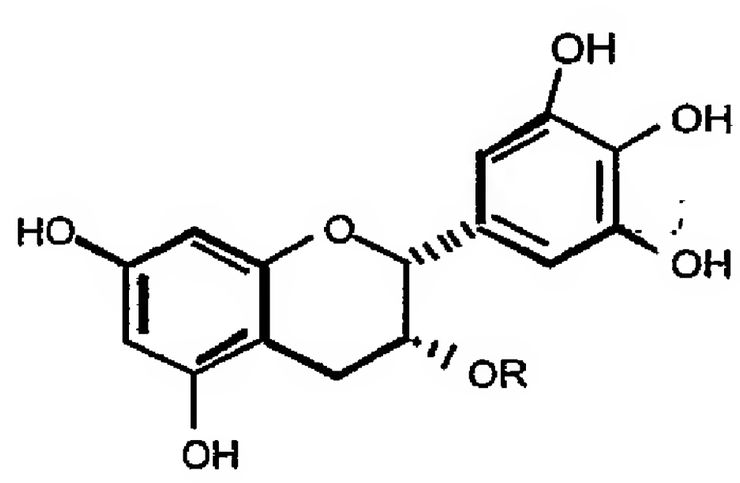


(IFb)

in which R is selected in the group of the following substitutive groups respectively:

(IFa)	(IFb)
R=H (-)-catechin [2*S*, *3R*]	R=H (+)-epicatechin [2*S*, *3S*]
R=gallate§	R=gallate§

Derivatives of (-)-epigallocatechin [2*R*, *3R*]



(IG)

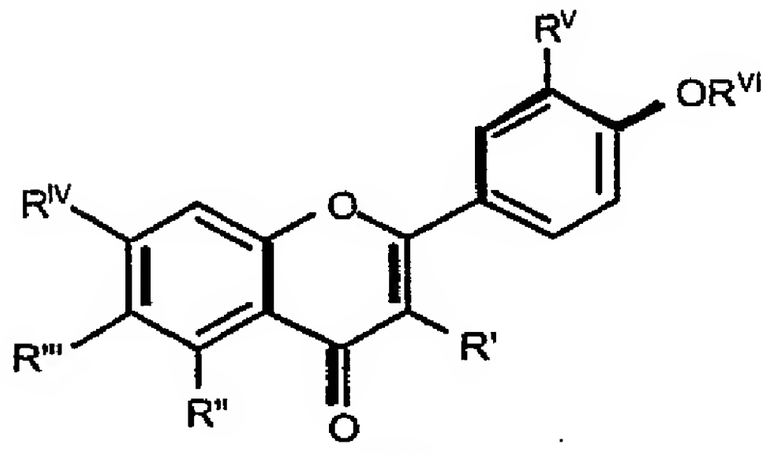
in which R is selected in the group of the following substituents: R=H; R=gallate§

In the above mentioned molecules the apici have the following meanings:

*natural compound; #synthetic molecule;

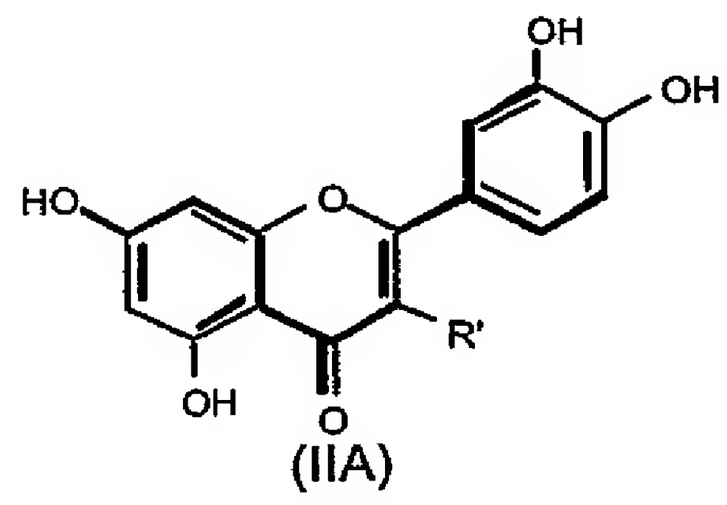
§molecule commercially available

Further, bibliographic references in which they are described are also reported.
The molecules represented by the general formula (II) are flavonic e flavonolic derivatives.



(II)

In which:
R' = H, OH, O-glycosidic portion that has a number of sugar residues ranging between 1 and 2, equal or different among them and bound each other, preferably chosen among β-D-glucose, β-D-galactose, β-D-xylose, α-L-ramnose, and corresponding mixtures;
R"=H, OH; R'"= H, OH, C-glucose; R^{IV} = H, OMe, O- glycosidic portion that has a number of sugar residues ranging between 1 and 2, equal or different among them and bound each other, preferably chosen among β-D-glucose, β-D-galactose, β-D-xylose, α-L-ramnose, and corresponding mixtures R^{V} = H, OH
R^{VI} = H, β-D-glucose.
Preferred molecules according to the general formula (II) are indicated with the following general formulas:
derivative of quercetin



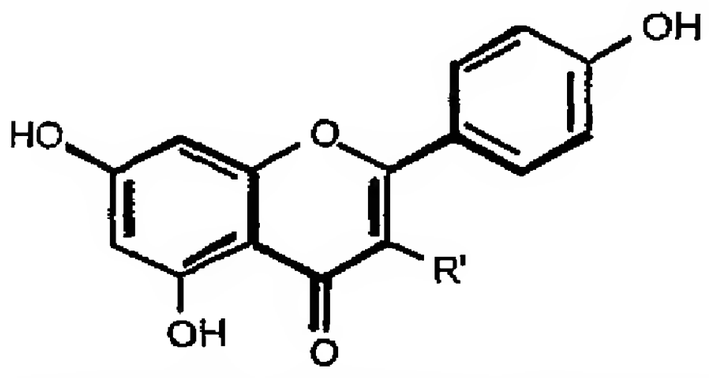
(IIA)

in which R' is chosen among one of the following substituents:

R'=OH, O-β-D-glucose, O-β-D-galactose, O-β-D-xylose, O-α-L-ramnose, O-β-D-glucose 6->1-α-L-ramnose (the arrow between the two units of glucose shows the binding with the next sugar)

Derivative of canferol:

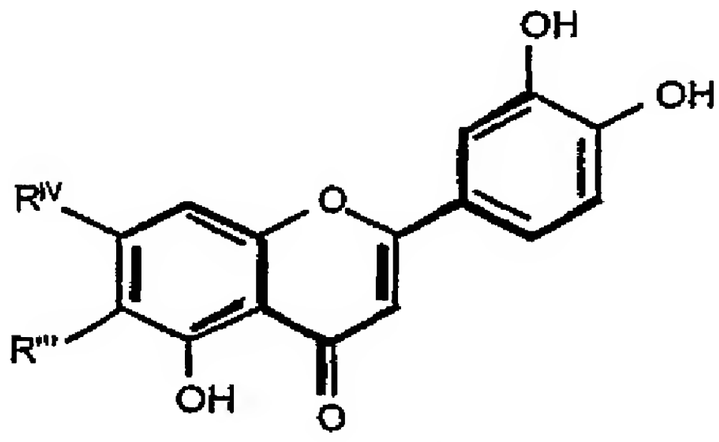
(IIB)



in which R is chosen between one of the following substituents:

R' = OH, O-β-D-glucose, O-β-D-galactose, O-β-D-xylose, O-α-L-ramnose, O-β-D-glucose 6->1-α-L-ramnose (the arrow between the two units of glucose shows the binding site with the next sugar).

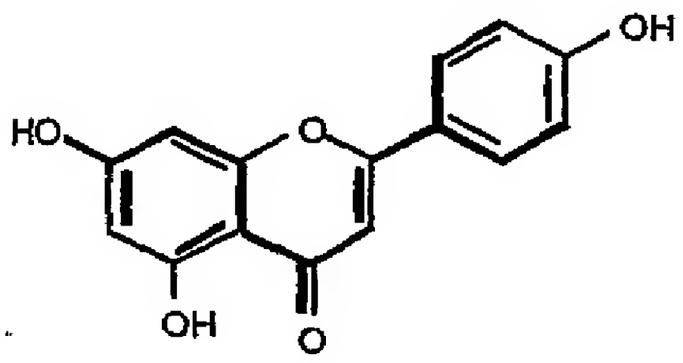
Derivative of luteolin



(IIC)

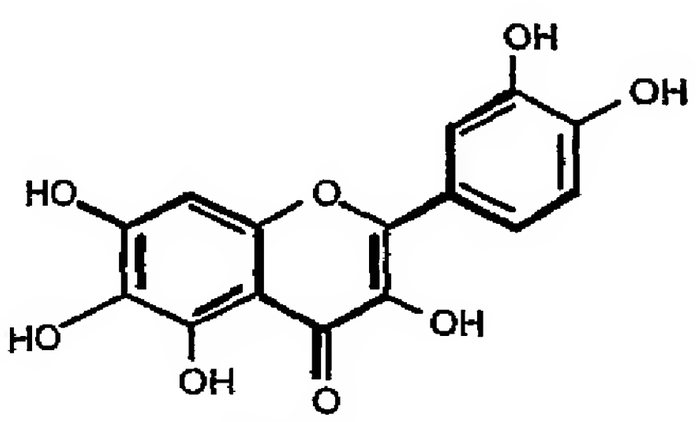
In which: R''' = H, OH, C-β-D-glucose, C-β-D-glucose-2->1-O-α-L-ramnose R^{IV}= OH, O-β-D-glucose

Apigenin:



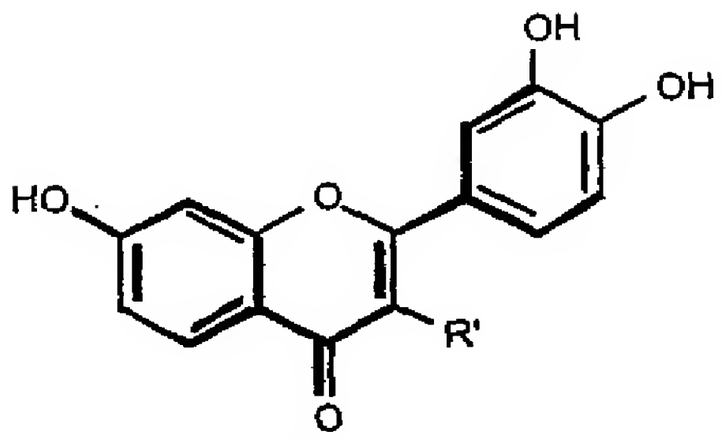
(II D)

Quercetagetin:



(II E)

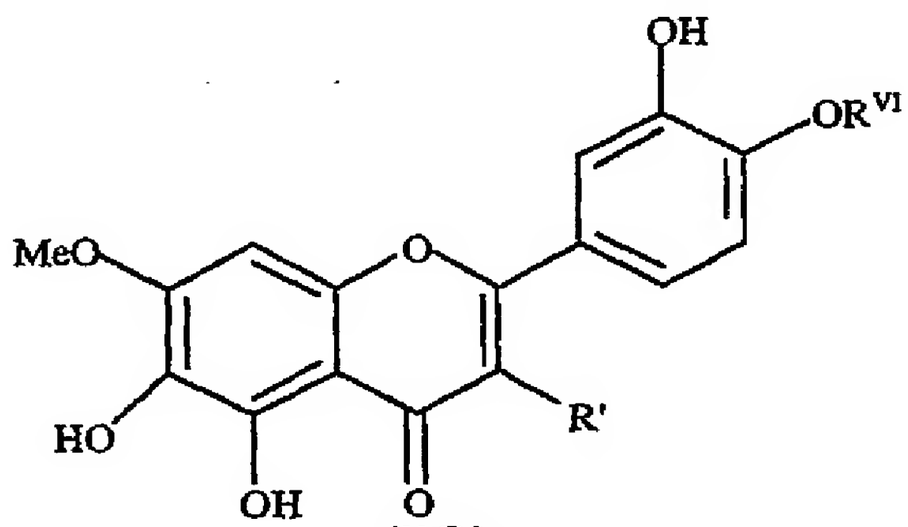
Derivative of fisetin



(IIF)

in which R' is chosen among one of the following substituents:

R' = OH, O-β-D-glucose, O-β-D-galactose, O-β-D-xylose, O-α-L-ramnose.



(IIG)

Preferred molecules according to the formula (IIG) are molecules in which:

R'=OH , R^{VI} = H;

R' = O-β-D-glucose, R^{VI} = H;

R' = OH, R^{VI} = β-D-glucose;

R' = O-(β-D-glucose1 -> 4-O- β-D-glucose) (the arrow between the two units of glucose shows the binding site with the next sugar), R^{VI} = H;

R' = O-(α-L-ramnose1 -> 2-O- β-D-glucose) (the arrow between the two units of glucose shows the binding site with the next sugar), R^{VI} = H;

R' = O-[(2-caffeoil)- β-D-glucose1 -> 4-O- β-D-glucuronic] (the arrow between the two glucosidic units shows the binding site with the next sugar), R^{VI} = H.

All compounds specified, in their diastereoisomers and/or optically active pure forms and their relative mixture are part of this invention.

The molecules of the general formulas (I) and (II) may be synthesized, for example, starting from the corresponding flavonoidic aglicons according to standard procedures of organic chemistry or can be purified from plants as, e.g.,

Anadenanthera macrocarpa, *Potentilla viscosa*, *Calliandra haematocephala*, *Guibourtia coleosperma*, *Paepalanthus latipes* and *Paepalanthus velloizioides* with standard extraction procedures or can be obtained commercially.

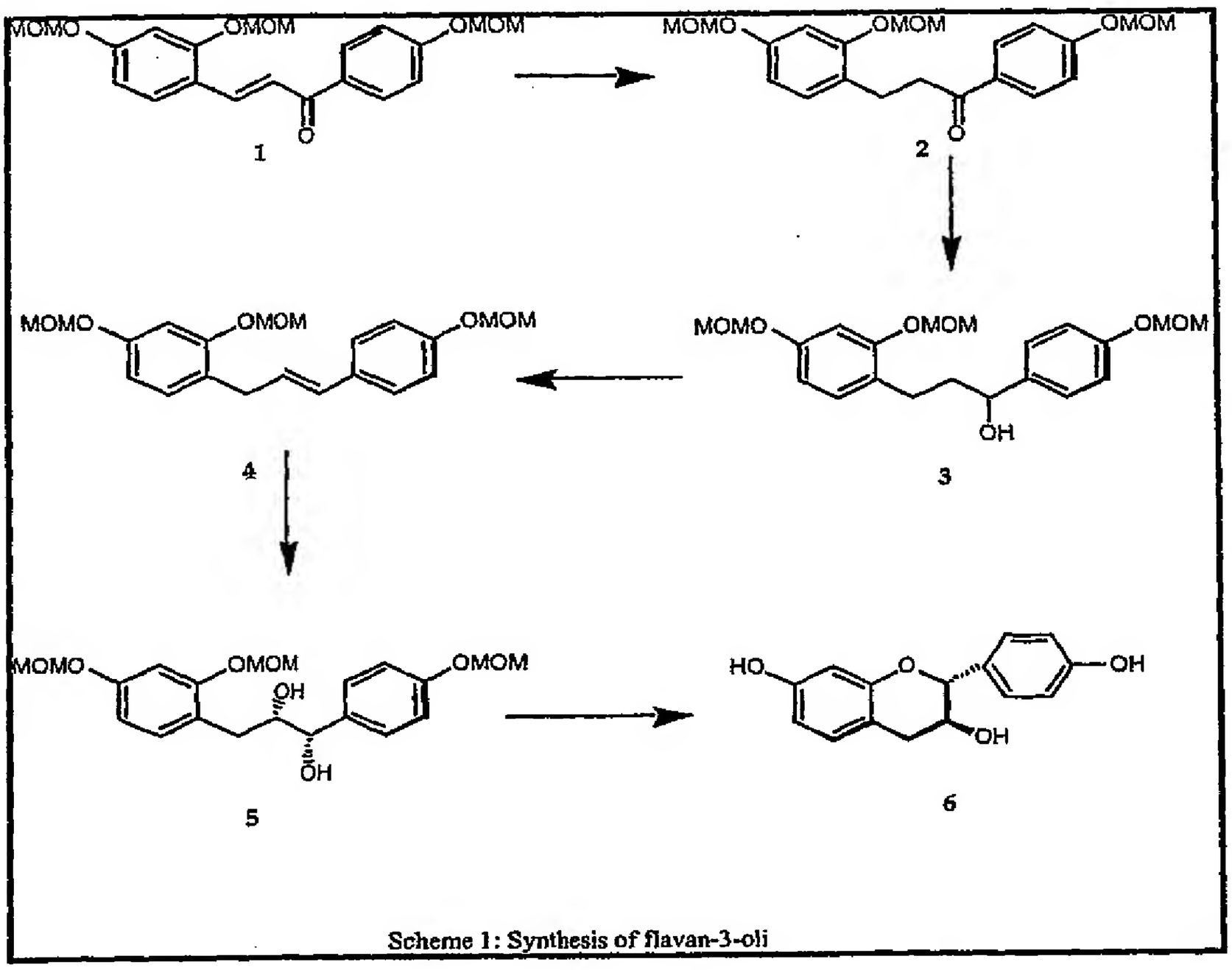
There are also included in the present invention the flavonoidic compounds extracted from plants, algae and sea weeds that contain them. Such extraction products can also be utilized according to the aim of this invention to modify MPS and induce stress genes. They can be obtained by using standard procedures and generally are flavonoid mixtures that can be used as such or following different steps of purification to separate more pure compounds that have particular biological interest.

Regarding molecules of formula (I), some of them, particularly aglicons (+)-catechin (2*R*,3*S*) and (-)-epicatechin (2*R*,3*R*), can be isolated from several plants or commercially available (e.g. Sigma) as starting material for related products. For example, the green tea (*Camelia sinensis*) is the main source of (-)-catechin (2*S*,3*R*), (-)-catechin-3-gallate (2*S*,3*R*), (+)-epicatechin (2*S*,3*S*), (-)-epicatechin-3-gallate (2*R*,3*R*). On the other hand, only occasionally efzelechin (2*R*,3*S*), fisetinidol (2*R*,3*S*) and guibourtinidol (2*R*,3*S*) are isolated from natural sources. Particularly, molecules of formula (II), can be isolated from the following Brazilian plants: *Paepalanthus latipes* and *Paepalanthus velloizioides* (Eriocaulaceae) (Vilegas et al 1999), as indicated in the examples.

The extraction can be performed on vegetable material, better if first dried. Several extraction steps are performed with solvents such as ether, chloroform, methanol, water and corresponding mixtures, that are later removed, generally by evaporation. The extracted material, redissolved in an appropriate solvent, is further fractionated by column chromatography . The eluted products are collected and characterized.

A general procedure of synthesis that can be used to prepare aglicons from flavan-3-oli includes the dioxydrilation of 1,3-diarilpropen, followed by acid-catalyzed cyclization, that produces diastereoisomers, according to procedures reported in the literature (Scheme 1), Nel *et al.* (1999). The hydrogenation of calcon (1) (*E*)-1-(4'-*O*-metoxymetylfenyl)-3-(2",4"-di-O-metoxymetyl-fenyl)-propenon in the presence of Pd/C produces *retro*-dihydrocalcon (2) that, after reduction with $NaBH_4$, produces 1,3-diarilpropan-1-olo (3) that is converted in (E)-

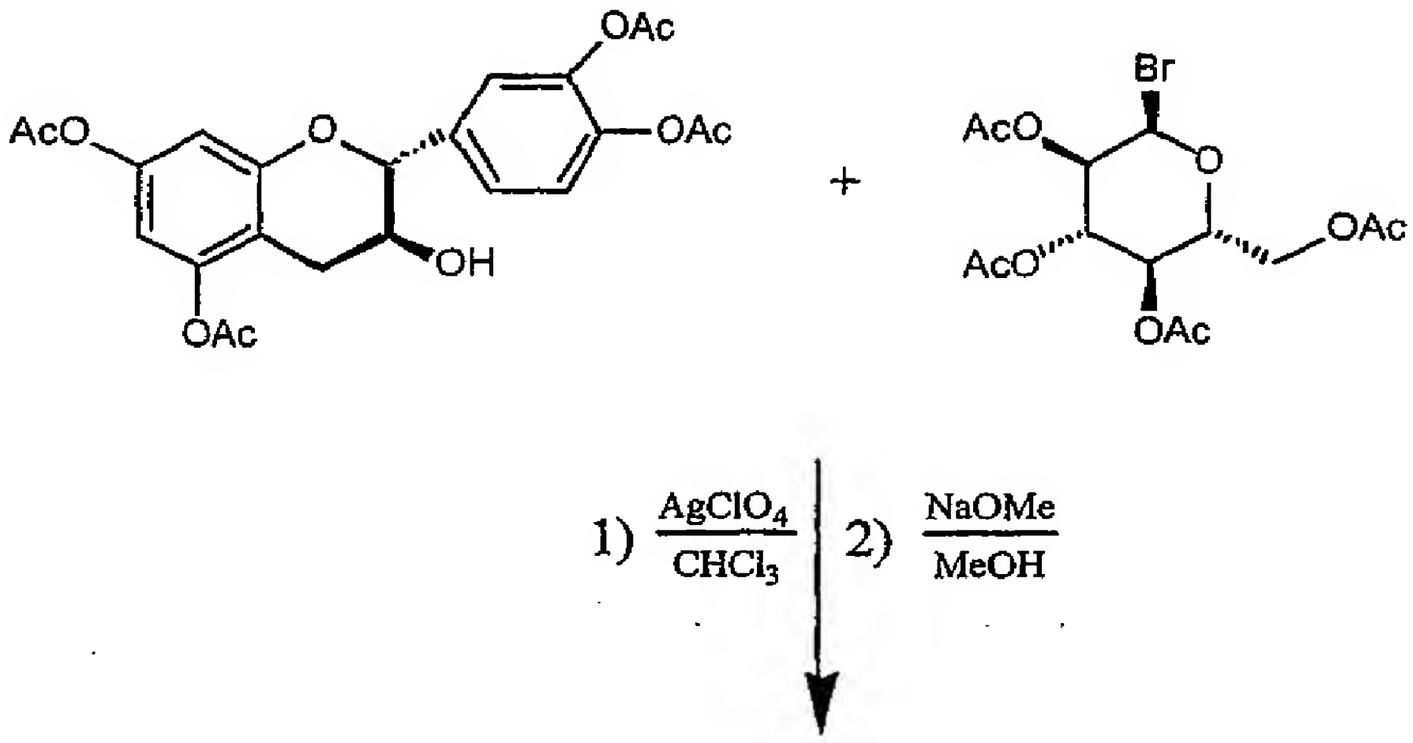
1,3-diarilpropen (4) using $SOCl_2$ and 1,8-diazabicyclo[5.4.0]undec-7-ene (1,8-DBU). From compound (4) by shaking in difasic system BuOH: H_2O 1:1 sin-diol is produced (5) by treatment with $MeSO_2NH_2$ at 0°C that, after deprotection and cyclization, produces the flavanic derivative (6).



Scheme 1: Synthesis of flavan-3-oli

Flavan-3-olo, acetylated in the aromatic –OH according to known organic reactions, can interact with the bromide of the peracetylated sugar to produce the corresponding glycoside.

A general procedure of synthesis that can be used to produce flavan-3-O-glycosids includes the initial synthesis of the appropriate aglicon in the aromatic –OH followed by the reaction of this with the halide of the sugar that had previously peracetylated. The so obtained compound is then desacetylated (scheme 2).



Scheme 2: Synthesis of glycosides

The molecules of formula (I) and (II) according to this invention and the corresponding pharmacologically acceptable salts and/or derivative, and the corresponding molecules in their diastereoisomer and/or optically active pure forms and corresponding mixtures, can be used in pharmaceutical applications, particularly in dermatology and cosmetics. It has been observed that such molecules have biological activity on membranes modifying their physical state. Thus, such compounds can be active in all those clinical diseases that are established when the MPS is altered and membranes are less functional under stress condition: oxidative stress, mechanic stress, osmotic stress, stress due to hypoxia, ischemia, heat shock, radiation shock, shock produced by toxic

compounds and free radicals, in degenerative chronic diseases and in the protection from cardiac and cerebral ischemia.

Thus, according to a characteristic of this invention compounds of the formula (I) and (II) can be used to treat: chronic degenerative illness, cardiac cerebral ischemia, diabetes, vascular and cardiovascular diseases, coronary and cerebral diseases, in allergies, immune and autoimmune diseases, of viral or bacterial origin, tumors, skin, mucosal, epithelial, renal diseases, traumas, neurodegenerative diseases, dementia, Alzheimer, Parkinson, epilepsy, AIDS, physiological stresses, ulcers, dermatitis, psoriasis, burns, etc.

According to an other characteristic of this invention compounds of formula (I) and (II) can be used to modify MPS of eukaryotic cells, particularly of animal, plant cells and of microorganisms and in particular of higher organisms and of human, with preventive and therapeutic uses.

Therefore, it is an aspect of this invention a method to modify MPS and to induce heat shock gene transcription including the treatment of the same cells with pharmaceutically acceptable amounts of the compounds of this invention.

Animal, mammalian and plant cells, or of microorganisms, are exposed to heat shock for different length of time (from 5 min to 2 hours or more) and at the same time or subsequently treated with the compounds of this invention. Such treatment causes a change in the MPS of membrane and accumulation of stress proteins.

Alternatively, the treatment includes exposure to the compounds of this invention and treatment with heat shock.

Preferably, treated cells are eukaryotic cells of plant or animal sources, in particular of mammals, more specifically human.

An evaluation of the biological activity of the compounds of the invention can be performed as follows.

The molecular assay according to this invention has been performed using described techniques of molecular biology as for example described in Sambrook et al. (2001), using suitable vectors harboring promoters that can express reporter gene(s) of interest (e.g. a human *hsp70* promoter) after exposure to stress (e.g. heat shock in mammalian or human cells, fibroblasts and/or keratinocytes). The identified substances are capable to induce *hsp70* gene transcription. Further, the method includes active molecules capable to modify MPS of the same cells or of

artificial lipidic membrane. It is thus possible to test their cosmetic effects, dermatological and pharmacological effects of the molecules under test in animal models and human clinical trials.

The molecular method used in this invention can be sketched in the following main steps:

- preparation of a suitable vector (e.g. that described in fig. 1) that harbors the *reporter* gene coding for a luciferase (or GFP, *green fluorescent protein*) under the control of an inducible *hsp70* promoter by heat shock in mammal or human;
- genetic transformation of mammalian or human cell lines with these constructs;
- assay of the protein product (luciferase or determination of GFP fluorescence) after heat shock;
- determination of anisotropy of the cell lines to determine changes in the MPS of the same cells or in artificial membranes.

Alternatively, rather than using a *reporter* gene, it is possible to use transcription of heat shock gene by Northern blot directly measuring the increase in *hsp70* mRNA accumulation, or by quantitative PCR.

For example, L929 cells, incubated at 37°C, are stressed by heat shock at 40° or 41°C with exposures variable from 20 min to an hour or more. After heat shock, a molecular assay that involves mRNA purification, its separation on agarose or acrylamide gels followed by hybridization with a labeled probe (e.g. *hsp70*, *hsp17* etc.) is performed. Alternatively, the activity of luciferase used as reported gene under the control of a heat shock promoter can be used (fig. 1). In this case, after heat shock cells are lysed in the presence of an appropriate substrate for luciferase, the activity is measured with a luminometer. We have identified and purified several molecules (listed in Table 1), from plants or chemically produced as described earlier, that are capable to induce a heat shock response higher or equal to that of Bimoclomol™ (utilized as an internal positive control, fig. 2).

The test utilized to assay the over expression of heat shock genes is based on a rapid enzymatic assay to determine luminescence of eukaryotic cells transfected with luciferase gene. The luciferase gene has been cloned in an eukaryotic plasmid under the control of human *hsp70* gene and transfected in murine fibroblasts (L929 cell line). In such cells, a heat shock induces *hsp70* promoter that activates transcription of the downstream gene (*reporter* gene, e.g. luciferase)

whose activity is measured determining luciferase in the presence of luciferine with a luminometer. L929 cells are treated with each of the molecules listed in figure 2 at a concentration of 10 μM and immediately exposed to a heat shock at different temperatures. After lysis, luciferase activity has been determined measuring the quantity of light emitted with a luminometer. With this assay it is possible to analyze rapidly the potential activity of several molecules. An additional negative control was established with molecule #100 (resveratrol) that inhibits the inducibility of *hsp70* mRNA transcription by heat shock. Similar assays measuring *hsp70* mRNA transcription was induced by heat shock, in constructs in which the luciferase gene had not been cloned, as a result of the exposure to the mentioned molecules can be established and measure by Northern blot.

All the molecules with heat shock mRNA inducibility are under the control of *hsp70* promoter (or to inhibit) have been evaluated in relation to their capacity to perturb MPS.

The assay that shows the capacity to modify membrane fluidity has been performed on artificial membranes (LUVs), made of di-oleil-fosfatidil-ethanolamine, di-oleil-fosfatidil-coline, cardiolipin and fosfatidylserine, that mimic the membrane lipid composition. In these membranes, every 500 lipid molecules is inserted. 1 molecule of DPH This substance emits fluorescent light when it is excited with polarized light. The more a membrane is rigid the less is the capacity of the molecules to rotate freely in the membrane. Using a fluorimeter that detects the emitted fluorescence by DPH, either vertically in respect to the polarizing light or horizontally, the anisotropy of the membrane is determined and correlated to its fluidity. The more the membrane is fluid, the more DPH rotates freely and emits light in a direction different from that with which it receives the excitation. To a higher value of anisotropy corresponds a higher rigidity of the membrane, while a lower anisotropy (low fluorescence) corresponds a higher fluidity. The example with molecule #11 (IC containing -β-D-xylose in position 3 that is anadentoside, (+)-fisetidinol [2R,3S] 3-β-D-xylose) and with #100 (resveratrol) show that molecules that fluidify biological membranes also induce a heat shock response, while a membrane rigidification corresponds inhibition of heat shock genes, or of genes controlled by a heat shock promoter.

A further aspect of this invention is the preparation of pharmaceutical compositions that include molecules of formula (I) and (II), either optically active and/or containing diastereoisomerically pure molecules or in mixtures, as salts and/or as derivatives, all of them pharmaceutically active, that can be easily synthesized by the expert in the field. These compositions can be prepared by known methodologies, by mixing the active principle preferably in a concentration between 0.1 and 99.5 % in weight with other components. The other components of the mixture can advantageously contain, also in combination, non active ingredients such as: eccipients, diluents, stabilizers, or other adjuvants such as to obtain compositions administrable orally, parenterally, rectaly, topically, spray. For example, as pills, tablets, capsules, granules, syrup, solutions, suspensions, creams, ointments, gels, powders, controlled or retarted formulations.

The kind of mode of administrations and dosage and quantities will depend on the type of disease and kind of formula used. The composition of creams for topical use in cosmetics are particularly preferred. They can be prepared according to known techniques that mix the active principle(s) with other ingredients.

The following examples are presented to show the invention and are not to be considered limitative of its scope.

Materials and methods

The chemicals used were pure products by Aldrich, Fluka, Carlo Erba, Sigma, Stratagene, Clontech, Amersham, etc.

The instruments used are routinely used in chemistry, analyses of molecules, DNA sequencer, NMR, luminometer, spectrophotometers, electrophoresis apparatuses, etc

Example 1

Synthesis of guibourtinidol (2R,3S, molecules ID) and its diastereomers

The synthesis (as referred to scheme 1) involves the following steps: condensation (performed according to Nel et al. 1999) of 2,4-di-O-metoxymethylbenzaldehyde and 4-O-metoxymethylaceto-fenone with yields of ca. 70% producing (E)-retro-2,4,4'-tri-O-metoxymethylcalcone (1).

The quantitative hydrogenation of (1) in presence of 5% carbon palladiate produces (2). Reduction of (2) with Na BH_4 synthesizes 1,3-diarylpropane-1-olo

(3), converted into (E)-1,3-diarylpropene (4) with a yield of ca. 60% using $SOCl_2$ and 1,8-DBU (1,8-diazobicycl [5.4.0] indec-7-ene). Treatment of (4) with $MeSO_2NH_2$ in a biphasic system $BuOH/H_2O$ (1:1) produces 1S,2S-sin-diolo (5) with a yield of ca. 70%. Simultaneous deprotection and cyclization of (5) with 3 M HCl in methanol at 60°C produces (2*R*,3*S*)-2,3-trans-4',7-diidroxyflavan-3-olo (6) (60% yield) and (2*S*,3*S*)-2,3-cis-4',7-diidroxyflavan-3-olo (7) (20% yield).

Example 2

Compound IA: (+)-catechin

(+)-catechin (and/or (-)-epicatechin) acetylated with acetic anhydride in pyridine produces 3',4',5,7-tetra-O-acetyl-(+)-catechin (and 3',4',5,7-tetra-O-acetyl-(-)-epicatechin respectively) that, treated with tetra-O-acetyl-β-D-glucopyranosilbromide, produces 3-O- β-D-glucopyranoside peracetylated (scheme 2). The latter, after saponification with sodium metoxyde in methanol, generates respectively (+)-catechin-3-O- β-D-glucopyranoside (or Ia(-)-epicatechin-3-O- β-D-glucopyranoside). The yields of the reaction can be increased utilizing the procedure for the synthesis as described by Sasuga et al 2000, using silver perchlorate and silver trifluoromethansulfonate as condensing agent.

Example 3

Extraction of fisetinidol-3-O- β-D-xylopiranoside (anadentoside)

Fisetinidol-3-O-β-D-xylopiranoside has been isolated by the bark of *Anadenanthera macrocarpa* (Leguminosae), a South Americanan vegetable species (Bolivia) (Piacente et al 1999).

The dried vegetable material (ca. 300 gr) has been initially extracted with ether and then with chloroform (1.4 gr). The same material then has been extracted with a mixture chloroform-methanol 9:1 (4.0 gr). The extracted material, dissolved in methanol, has then been fractionated on a Sephadex LH-20 column (Pharmacia). Collecting fractions of ca. 10 ml, fractions 50-58 contained 25 mg of compound IC with R=β-D-xylose. The latter has been characterized by nuclear magnetic resonance and mass spectrometry as reported by Piacente et al 1999.

Example 4

Characterization of compound fisetinidol-3-O-β-D-xylopiranoside .

The molecular formula ($C_{20}H_{22}O_9$) was determined with experiments using ^{13}C NMR, ^{13}C DEPT NMR and FAB-MS in negative ions, the compound showed a quasi-molecular peak $[M-H]^-$ at m/z 405 and a fragment at m/z $[(M-H)-132]^-$ due to the loss of a pentose unit. The spectrum ^1H NMR in the aromatic region showed signals a δ 6.72 (1H, dd, *J* = 2.0 and 8.3 Hz, H-6'), 6.76 (1H, d, *J* = 8.3 Hz, H-5') and 6.82 (1H, d, *J* = 2.0 Hz, H-2') due to the presence of the ring B 1',3',4'-trisubstitued of a flavanoidic skeleton and signals at δ 6.33 (1H, d, *J* = 2.0 Hz, H-8), 6.36 (1H, dd, *J* = 2.0 and 8.3 Hz, H-6) and 6.85 (1H, d, *J* = 8.3 Hz, H-5) in agreement with the presence of a single oxydrilic group at C-7 in the ring A. Also evident were signals at δ 2.82 (1H, dd, *J* = 6.2 and 15.6 Hz) and 2.87 (1H, dd, *J* = 4.8 and 15.6 Hz), typical of H_2-4 of a flavanic derivative and at δ 3.10 (1H, dd, *J* = 7.3 and 8.7 Hz), 3.15 (1H, t, *J* = 11.4 Hz), 3.23 (1H, t, *J* = 8.7 Hz), 3.45 (1H, ddd, J = 6.2, 8.7 and 11.4 Hz), 3.85 (1H, dd, J = 6.2 and 11.4 Hz), 4.15 (1H, m), 4.16 (1H, *d*, J = 7.3 Hz) and 4.97 (1H, *d*, J = 5.9 Hz) all of which that can be attributed to protons with alcoholic functions. The DQF-COSY spectrum showed the CH_2 (δ 2.82 and 2.87)-CHOH (δ 4.15)-CHOH (δ 4.97) sequence due to the presence of an aliphatic eterocyclic ring of a flavanol and the typical system of spin of β-D-xylopyranose. In particular, the coupling constants of the signals that can be attributed to H-2 (*J* = 5.9 Hz) and to H-3 (J = 4.8, 5.9 and 6.2 Hz) of the aglicon suggested a C-2 and C-3 with the same stereochemistry of (+)-catechin. The HSQC experiment, that correlates the protonic signals to the corresponding carbonic signals, allowed to establish the presence of a shift due to the glycosidation at C-3 of the aglicon (δ 76.9), allowing to infer that the residue of xylose was bound to C-3. The HMBC spectrum that showed the correlation between the protonic signals at δ 2.82 and 2.87 and C-10 (δ 112.4), C-5 (δ 131.5), C-9 (δ 155.9), the protonic signals at δ 4.15 and C-2 (δ 80.7), between the protonic signals at δ 4.97 and C-1' (δ 132.2), C-2' (δ 114.8) and C-6' (δ 119.6) allowed us to assign the resonances of the quaternary carbons and to infer for the aglicon of IC, the structure of 3,3',4', 7-tetrahydroxyflavan (fisetinidol). An additional correlation was observable between the signal of the anomeric proton at δ 4.16 and C-3 (δ 76.9) of fisetinidol. On the bases of such data to the compound IC with R=β-D-

xylose has been assigned the structure fisetinidol-3-O-β-D-xylopyranoside, called anadantoside.

Example 5

Isolation of derivatives of 7-O-methylquercetagetin - general formula (IIG)

Inflorescences and pulverized leaf powder (100 g in both cases) of *P. latipes* and *P. velloizioides* were extracted with chloroform and then with 80% methanol (steeping at room temperature, 1 week for each solvent). Solvents were then vaporized under vacuum, followed by collection of the solid material. The concentrated methanolic extracts of each plant were dissolved in water, filtered and chromatographed on a XAD-2 column with 3 liters of water and then with 1 liter of methanol. An aliquot (ca 1.2 g) of the methanolic extract of *P. latipes* was separated on a Sephadex LH-20 (80 x 2 cm). Fractions of .ca 8 ml were eluted with methanol and checked by TLC using as eluant buthanol/acid acetic/water (13:3:5). Fractions 31-36 and 41-44, obtained with the Sephadex, were further purified by HPLC Waters μ-Bondapak RP-18 (30 cm x 7.6 mm i.d.) column using methanol/water (9:11) as eluant; from fractions 31-36 we obtained in pure form, compounds with R'= β-D-glucose 1->4 β-D-glucose and R"=H; with R'=β-D-glucose and R"=H; from fractions 41-44 we obtained in pure form compounds con R'= 6-caffeoil-β-D-glucose 1->4 β-D-glucoronic and R"=H, fractions 51-56 contained, in pure form, compound R'=H and R"=β-D-glucose and fractions 67-70 contained, pure, compounds R'=R"H; The methanolic extract (1.2 g) of *P. velloizioides*, was chromatographed on a Sephadex LH-20 and on a HPLC with the previously described conditions; from fractions 33-40 we obtained in pure form, compounds II with R'= β-D-glucose 1->4 β-D-glucose and R"=H; II with R'=β-D-glucose and R"=H, from fractions 48-52 compound II with R'= α-L-ramnose 1->2 β-D-glucose and R"=H and from fractions 64-69 compound II with R'=R"=H.

Table 1

# C	Control
# BRLP	bimoclomol™
# 2.	quercetagetin-7-methyl ether
# 3.	quercetagetin-7- methyl ether-4'-O-β-D-glucopyranoside
# 4.	quercetagetin 7- methyl ether-3-O-neohesperidoside
# 5.	quercetagetin-7- methyl ether-3-O-β-D-glucopyranoside
# 7.	6-idroxyluteolin-7-O-β-D-glucopyranoside,
# 11.	fisetinidol-3-O-β-D-xylopyranoside
# 13.	luteolin-6-C-β-D-glucopyranoside
# 14.	luteolin-6-C-[α-L-ramnopyranosil-(1->2)-O]-β-D-glucopyranoside
#100.	Resveratrol

References

Bae, Y.S., et al.; *Phytochemistry* 35, 473-478 (1994)

Biro, K., et al.; G *NeuroReport*. 9: 2029-2033 (1998)

Banefeld, M., et al.; *Phytochemistry* 25, 1205-1207 (1986)

Carratu L, et al.; *Proc Natl Acad Sci USA* 93: 3870-3875 (1996)

Cossins A *Temperature adaptation of biological membranes*, Edited by Cossin, A.R., London, Portland Press, (1994)

Edwards MJ, et al.; *J Cutan Pathol* 26: 483-489, (1999)

Horvath I, et al.; *Proc Natl Acad Sci* USA. 95: 3513-3518 (1998)

Kamya S, Nakagawa, Y, Sasuga, K. Japanese Patent CAN: 121: 91330 AN 1994:491330.

Latchman, DS *Int. J. Mol. Med.* 2: 375-381 (1998)

Lindquist, S. *Ann. Rev. Biochem.* 55: 1151-1191 (1986)

Marber MS, Mestril R, Chi SH, Sayen MR, Yellon DM & Dillmann WH *J Clin Invest*, 95, 1446-56 (1995)

Maytin, EV *J Biol Chem* 267: 23189-96 (1992)

Morimoto RI and Santoro MG *Nat. Biotech*, 16: 833-38 (1998)

Morimoto, S., et al.; *Chem. Pharm. Bull.* 34, 633-642 (1986)

Okubo S, Wildner O, Shah MR, Chelliah JC, Hess ML, Kukreja RC *Circulation* 103:877-81 (2001)

Nel JJ Reinier, et al. *Phytochemistry* 52, 1153-1158 (1999)

Piacente, S., et al.; C. *Phytochemistry* 51, 709-711 (1999)

Plumier J-CL & Currie RW *Cell Stress & Chaperones* 1, 13-17 (1996)

Plumier J-Cl., Ross BM, Currie RW, Angelidis CE, Kazlaris H, Kollias G & Pagoulatos GN *J. Clin. Invest.* 95, 1854-60 (1995)

Rajdev S, Hara K, Kokubo Y, Mestril R, Dillmann W, Weinstein PR, Sharp FR *Ann Neurol* 47:782-91 (2000)

Sambrook J, et al.; *Molecular Cloning: A Laboratory Manual* Third Edition CSH Laboratory press, Cold Spring harbor, NY, , (2001)

Sammut IA, Jayakumar J, Latif N, Rothery S, Severs NJ, Smolenski RT, Bates TE, Yacoub MH *Am J Pathol.* 158:1821-31, (2001)

Santoro MG. *Biochem Pharmacol.* 59: 55-63 (2000).

Sasuga, K., et al.; *Chagyo, Kenkyu, Hokoku* 89, 29-36 (2000)

Singer MA, et al.; *Trends Biotechnol.* 16: 460-468 (1998)

Slater SJ, et al.; *J Biol Chem.* 269: 4866-4871 (1994)

Suzuki I, et al.; *EMBO J.* 19: 1327-1334 (2000)

Torok Z, et al.; *Proc Natl Acad Sci USA* 98: 3098-3103 (2001)

van Eden W et al.; *Stress Proteins in Medicine*, Eds., Marcel Dekker Inc. (1996)

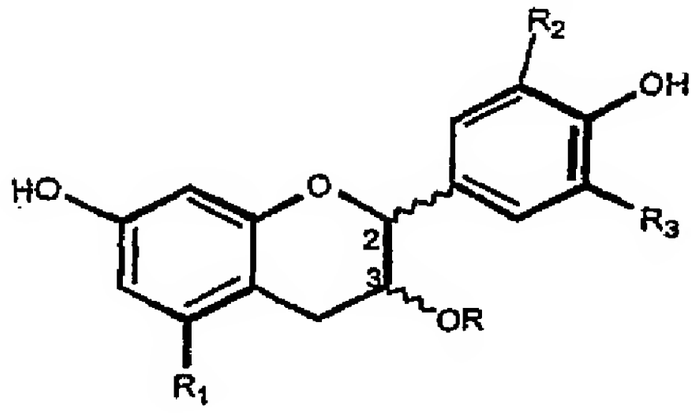
Vigh L, et al.; *Trends Biochem Sci.* 23: 369-374 (1998)

Vigh, L., et al.;*Nature Medicine* 3: 1150-1154 (1997)

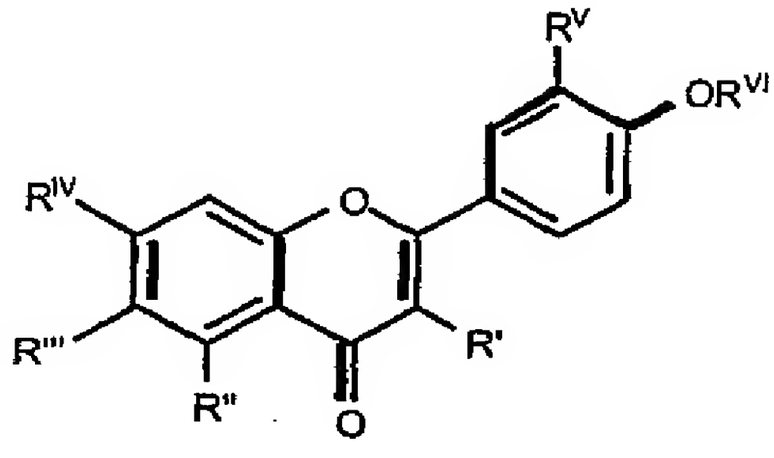
Vilegas, W., et al., *Phytochemistry* 51, 403-409 (1999)

CLAIMS

1. Flavonoidic compounds of the general formula (I) and (II):



(I)



(II)

to be used in the pharmaceutical field in which:

R = H, gallate, glicosidic portion having a number of sugar residues, equal or different among each other and bound one to the other, comprised between 1 and 2;

R1, R2, R3, equal or different among each other, are H or OH

R' = H, OH, O-glicosidic moiety carrying a number of sugar residues, equal or different among each other and bound one to the other, comprised between 1 and 2;

R'' = H, OH

R''' = H, OH, C-glucose

R^{IV} = H, OMe, O- glicosidic moiety carrying a number of sugar residues, equal or different among each other and bound one to the other, comprised between 1 and 2;

R^{V} = H, OH

R^{VI} = H, β-D-glucose.

2. Flavonoidic compounds of formula (I) according to claim 1 selected in the group of compounds having the following substitutes:

R=β-D-glucose, β-D-mannose, β-D-galactose, β-D-xylose, α-L-arabinose, β-D-quinovose, β-D-fucose, α-L-ramnose, and corresponding mixtures.

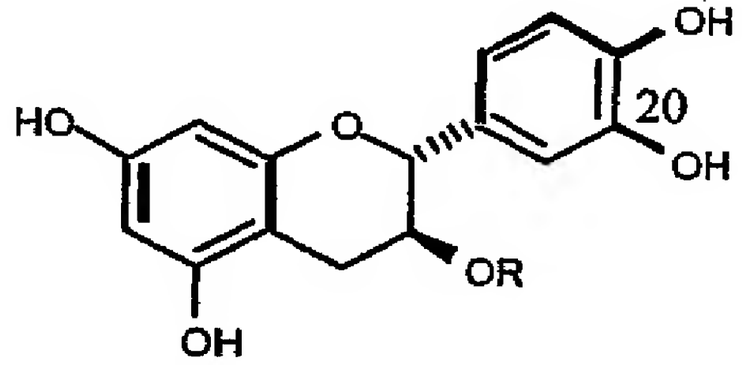
3. Flavonoidic compounds of formula (II) according to claim 1 selected in the group of compounds having the following substitutes:

R' = β-D-glucose, β-D-galactose, β-D-xylose, α-L-ramnose, and corresponding mixtures;

4. Flavonoidic compounds of formula (II) according to claim 1 selected in the group of compounds having the following substitutes:

R^{IV} = β-D-glucose, β-D-galactose, β-D-xylose, α-L-ramnose, and corresponding mixtures.

5. Compounds according to claim 1 that are derivatives of (+)-catechin [2*R*, 3*S*] according to the general formula (IA)

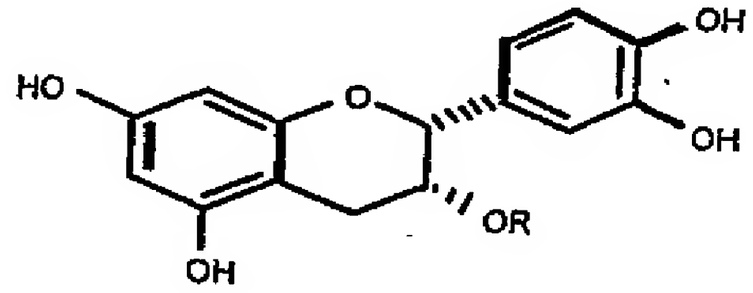


(IA)

in which R is selected in the group of compounds having the following substitutes:

R=H; R=-D-glucose; R=-D-mannose; R=-D-galactose; R=-D-xylose; R=-L-arabinose; R=-D-quinovose; R=-L-ramnose; R = gallate.

6. Compounds according to claim 1 that are derivatives of (-)-epicatechin [2*R*, 3*R*] according to the general formula (IB):

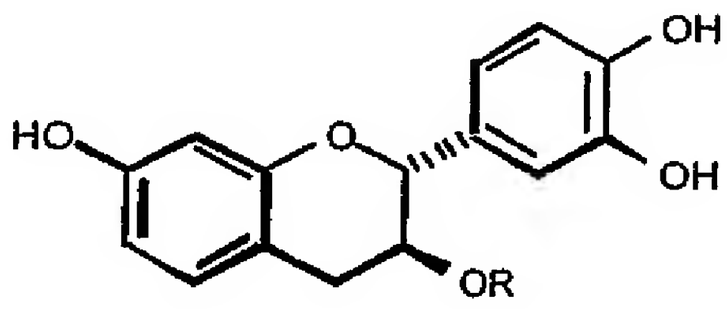


(IB)

in which R is selected in the group of compounds having the following substitutes:

R=H; R=β-D-glucose; R=β-D-mannose; R=β-D-galactose; R=β-D-xylose; R=α-L-arabinose; R=β-D-quinovose; R=α-L-ramnose; R=gallate.

7. Compounds according to claim 1 that are derivatives of (+)-fisetidinol [2*R*, 3*S*] according the general formula (IC)

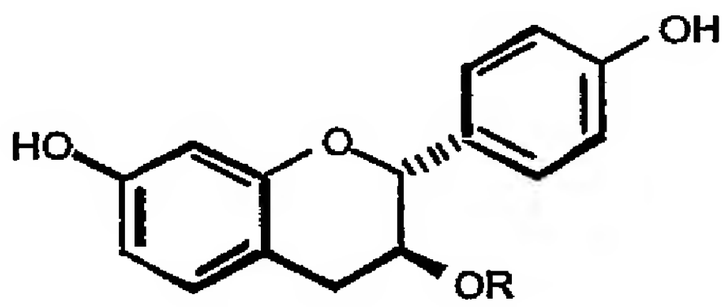


(IC)

in which R is selected in the group of compounds having the following substitutes:

R=H; R=-D-glucose; R=-D-mannose; R=-D-galactose; R=-D-xylose; R=-L-arabinose; R=-D-quinovose; R=-L-ramnose; R=gallate.

8. Compounds according to claim 1 that are derivative of (+)-guibourtinidol [2*R*, 3*S*] according the general formula (ID)

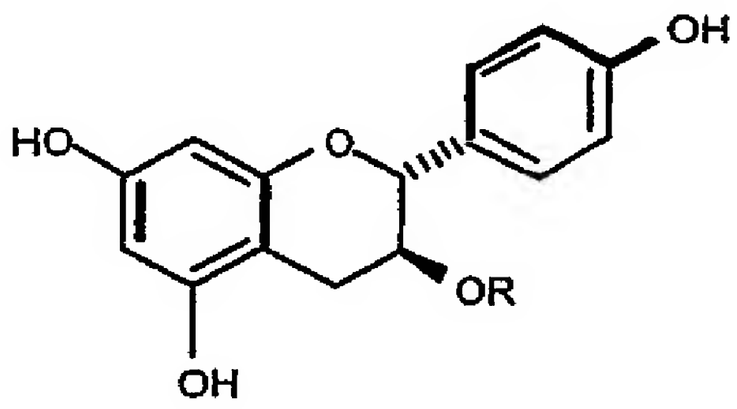


(ID)

in which R is selected in the group of compounds having the following substitutes:

R=H; R=-D-glucose; R=-D-xylose; R=-D-quinovose.

9. Compounds according to claim 1 that are derivatives of (+)-efzelechin [2*R*, 3*S*] according the general formula (IE)

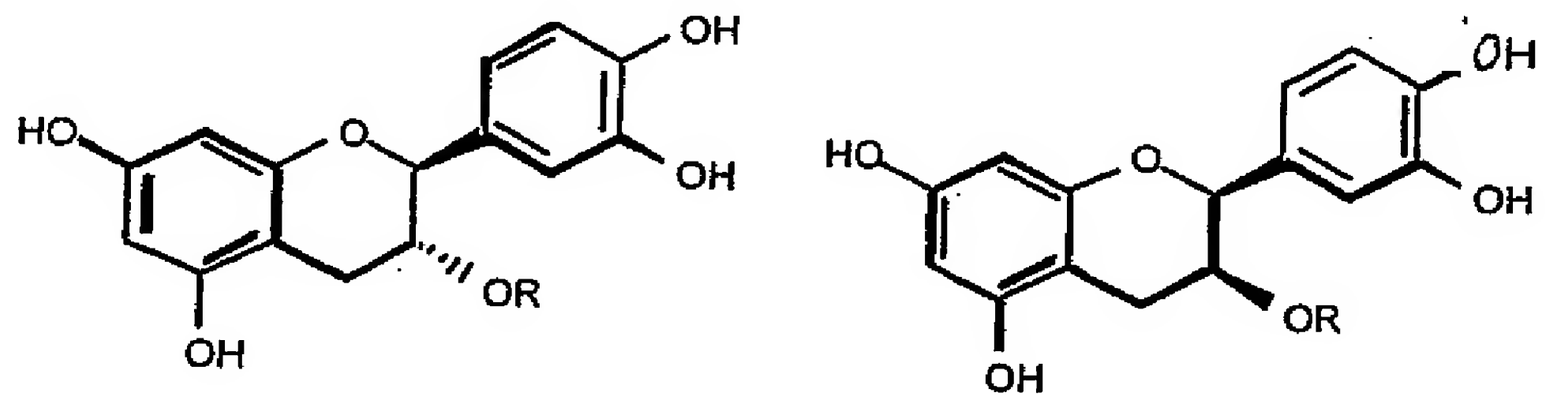


(IE)

in which R is selected in the group of compounds having the following substitutes:

R=H; R=-D-glucose; R=-D-xylose; R=-D-quinovose.

10. Compounds according to claim 1 that are derivatives of flavan-3-olo with stereochemistry 2*S* according to the general formula (IFa) and (IFb)



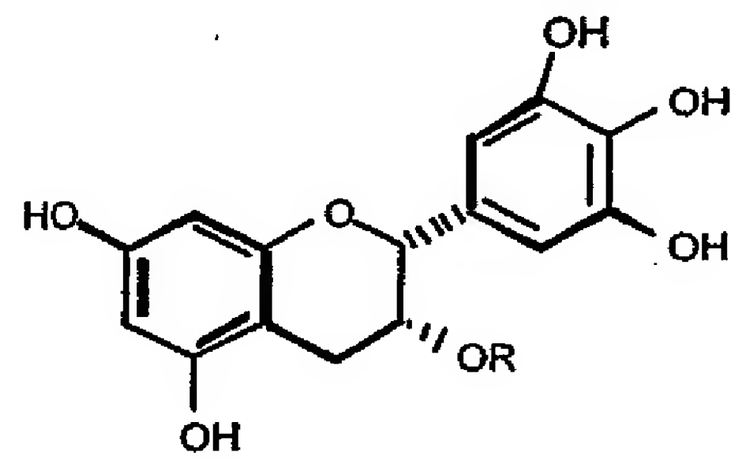
(IFa) (IFb)

in which R is selected in the group of compounds having the following substitutes of the two following substituents:

R=H (-)-catechin [2*S*, *3R*], R=gallate, according to (IFa);

R=H (+)-epicatechin [2*S*, *3R*], R=gallate, according to (IFb).

11. Compounds according to claim 1 that are derivatives of (-)-epigallocatechin [2*R*, *3R*] according to the general formula (IG)

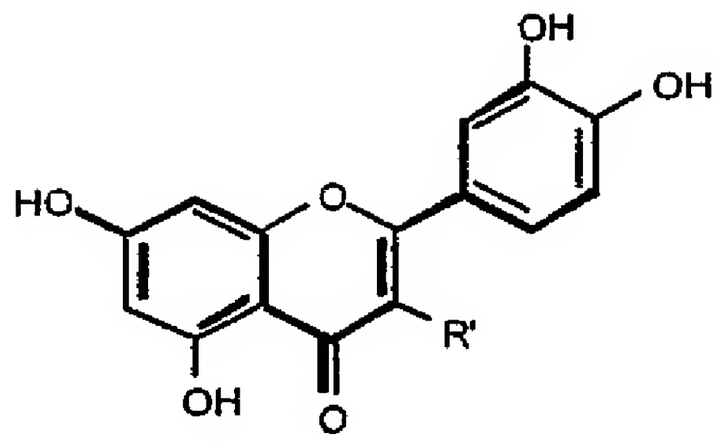


in which R is selected in the group of the following substitutes: R=H; R=gallate

12. Compounds of formula (I) according to claims 1-11 in which the atoms C in position (2) and (3) have the configuration R or S independently from each other.

13. Compounds according to claims 1-12 in which the OH groups are substituted by peracetilyc groups.

14. Compounds according to claim 1 that are derivatives of quercetin according to the general formula (IIA)

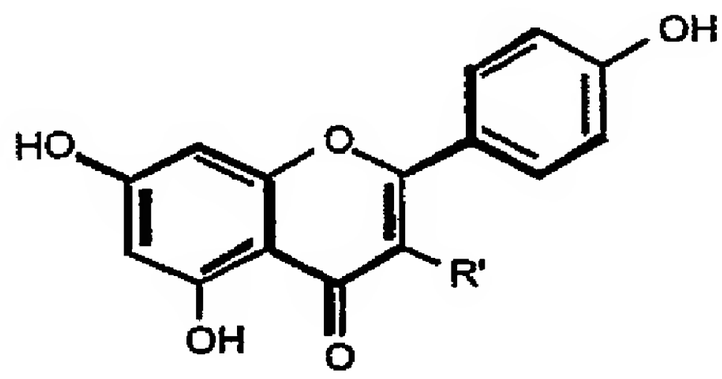


(IIA)

in which R' is selected in the group of compounds having the following substitutes:

R'=OH, O-β-D-glucose, O-β-D-galactose, O-β-D-xylose, O-α-L-ramnose, O-β-D-glucose 6->1-α-L-ramnose.

15. Compounds according to claim 1 that are derivatives of canferol according the general formula (IIB)

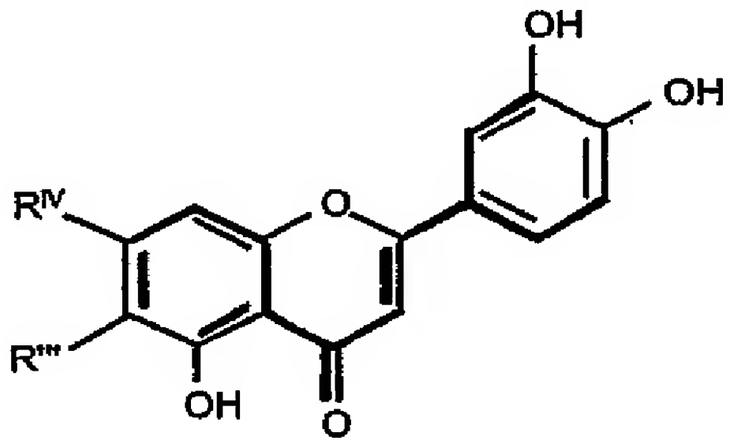


(IIB)

in which R' is selected in the group of compounds having the following substitutes:

R' = OH, O-β-D-glucose, O-β-D-galactose, O-β-D-xylose, O-α-L-ramnose, O-β-D-glucose 6->1-α-L-ramnose.

16. Compounds according to claim 1 that are derivatives of luteolin according the general formula (IIC)

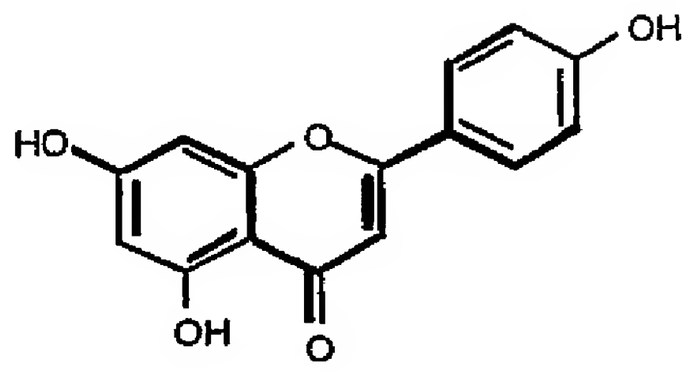


(IIC)

in which:

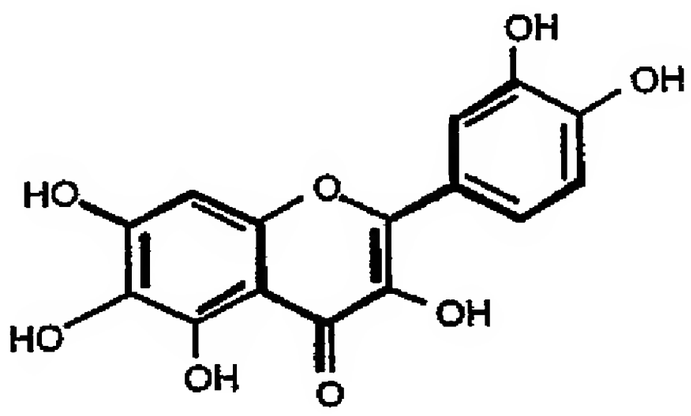
R''' = H, OH, C-β-D-glucose, C-β-D-glucose-2->1-O-α-L-ramnose R^{IV}= OH, O-β-D-glucose.

17. Compounds according to claim 1 that are derivatives of apigenin according to the general formula (II D)



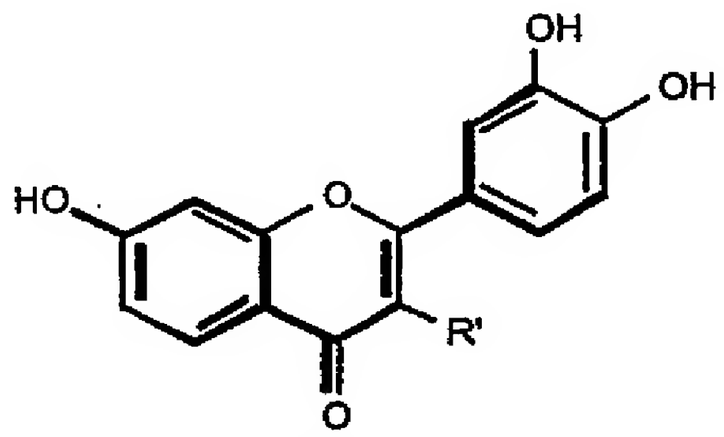
(IID)

18. Compounds according to claim 1 that are derivatives of quercetagetin according to the general formula (IIE)



(IIE)

19. Compounds according to claim 1 that are derivatives of fisetin according to the general formula (IIF)

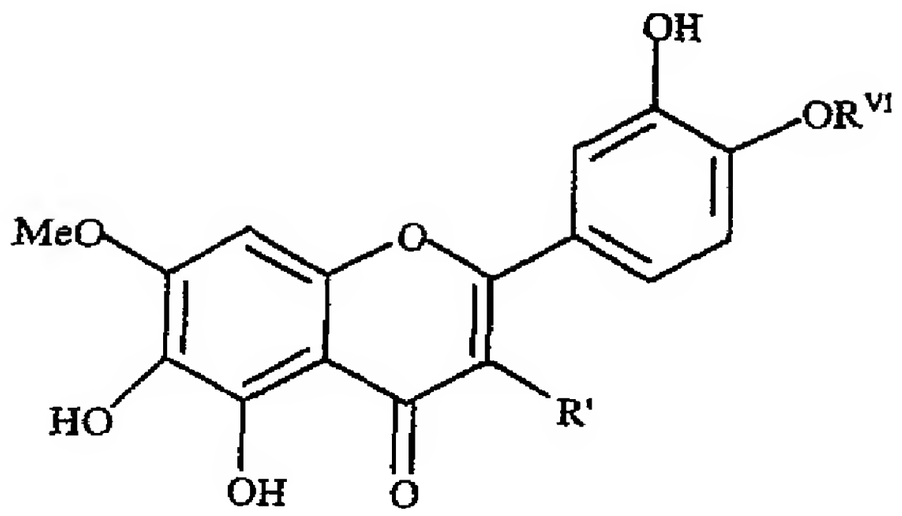


(IIF)

in which R' is selected in the group of compounds having the following substitutes:

R' = OH, O-β-D-glucose, O-β-D-galactose, O-β-D-xylose, O-α-L-ramnose.

20. Compounds according to claim 1 according to the general formula (IIG)



(IIG)

in which:

R'=OH , R^{VI} = H;

R' = O-β-D-glucose, R^{VI} = H;

R' = OH, R^{VI} = β-D-glucose;

R' = O-(β-D-glucose1 -> 4-O-(β-D-glucose), R^{VI} = H;

R' = O-(α-L-ramnose1 -> 2-O-(β-D-glucose), R^{VI} = H;

R' = O-[(2-caffeoil)- β-D-glucose1 -> 4-O-(β-D-glucuronic], R^{VI}= H.

21. Compounds according to claims 1-20 in their diastereoisomeric forms, pure and/or pure enantiomer.
22. Compounds according to claims 1-21 to be used for dermatological use.
23. Compounds according to claims 1-21 to be used for cosmetic use.
24. Compounds according to claims 1-21 to be used to modify MPS increasing the synthesis of stress proteins.
25. Flavonoidic compounds obtained by extraction and purification from plant material to be used for dermatological or cosmetic use to treat ailment and non-esthetic modifications correlated to a change in MPS of eucharíotic celles.
26. Products according to claim 25 in which the cells belong to higher animals, including mammals and human.
27. Products according to claims 25 and 26 in which the plant material is chosen among the following plants: *Anadenanthera macrocarpa*, *Potentilla viscosa*, *Calliandra haematocephala*, *Guibourtia coleosperma*, *Eriocaulaceae*, *Paepalanthus latipes*, *Paepalanthus velloizioides*, *Camelia sinensis*, and related mixtures.
28. Products according to claims 25 and 26 chosen in the following group: (+)-catechin (2*R*,3*S*), (-)-epicatechin (2*R*,3*R*), (-)-catechin (2*S*,3*R*), (+)-epicatechin (2*S*,3*S*), efzelechin (2*R*,3*S*), fisetinidol (2*R*,3*S*), guibourtinidol (2*R*,3*S*), and corresponding glycosids, (-)-catechin-3-gallate (2*S*,3*R*), (-)-

epicatechin-3-gallate (2*R*,3*R*), quercetagetin-7-metil ether, quercetagetin-7-methyl ether -3-O [2-O-caffeoil-β-D-glucopyranosil (1->4)-O-β-D glucuronopyranoside], quercetagetin-7- methyl etere-3-O-neohesperidoside, quercetagetin-7- methyl etere-4'-O-β-D-glucopyranoside, quercetagetin-7-methyl ether -3-O-β-D-glucopyranoside, 6-idroxyluteolin-7-O-β-D-glucopiranoside, luteolin-6-C-β-D-glucopyranosid, fisetinidol-3-O-β-D-xylopiranoside, luteolin-6-C-[α-L-ramnopyranosil-(1->2)-O]-β-D-glucopyranoside, and corresponding mixtures.

29. Pharmaceutical compositions comprising as active principle an effective amount of a compound according to claims 1-28, pharmaceutically acceptable corresponding to derivatives and/or salts, comprising the corresponding optically active compounds in their enantiomers and/or pure diastereoisomers and corresponding mixtures, to prevent or treat illness connected to a change in MPS of eukaryotic cells.

30. Compositions according to claims 29 in which the cells belong to higher animals, including mammals and humans.

31. Compositions according to claims 28 and 29 in which the amount of the active principle ranges between 0.1 and 99.5 % in weight.

32. Compositions according to claims 28-31 further comprising: eccipients, diluents, stabilizers, or other adjuvants such to obtain composition to be administered orally, parenterally, rectally, topically, spray.

33. Compositions according to claims 28-32 as pills, tablets, granules, syrup, solution, suspension, creams, ointments, gels, powder, delayed and retarded formulation.

34. Compositions according to claim 33 in a cream for topical use.

35. Cosmetic compositions comprising as an active principle an effective amount of a compound according to claims 1-28, corresponding pharmaceutically acceptable derivatives and/or salts, including the corresponding compounds optically active in their forms as enantiomers and/or pure diastereoisomers and relative mixtures, to prevent and/or treat illness connected with changes in MPS.

36. Compositions according to claim 35 in which the cells are those of higher organisms including mammals and humans.

37. Compositions according to claims 35 and 36 in which the amount of the active principle ranges between 0.1 and 99.5 % in weight.
38. Compositions according to claims 35-37 further comprising: eccipients, diluents, stabilizers, or other adjuvants.
39. Compositions according to claim 38 for topical use.
40. Process to obtain compounds according to claims 1-11 comprising the following steps: hydrogenation of the starting product calcon (1) (*E*)-1-(4'-*O*-metoxymethylfenil)-3-(2",4"-di-O-metoxymethyl-fenil)-propene in presence of Pd/C to obtain *retro*-diidrocalcon (2) that by subsequent reduction with $NaBH_4$, gives 1,3-diarilpropan-1-olo (3) that is converted in (E)-1,3-diarilpropen (4) using $SOCl_2$ and 1,8-diazabicicle [5.4.0]undec-7-ene (1,8-DBU); from compound (4) by shaking in a diphasic system BuOH: H_2O 1:1 the corresponding *sin*-diolo (5) is obtained that, by subsequent deprotection and cyclization, yields a un flavanic derivative (6).
41. Process according to claim 40 further comprising the step of acetylation of the flavanic derivatives (6) on the aromatic –OH with subsequent reaction with the halide of the chosen sugar, previously peracetylated, to obtain the corresponding glycoside.
42. Method to modify MPS of eukaryotic cells characterized in that the cells are treated with effective amounts of at least one compound or product chosen among those of claims 1-28 and related mixtures.
43. Method to induce an heat shock response, such ad heat shock, in eukaryotic cells characterized in that the cells are treated with effective amounts of at least one compound or product chosen among those of claims 1-28 and related mixtures.
44. Method to protect eukaryotic cells from stress conditions characterized in that the cells are with effective amounts of at least one compound or product chosen among those of claims 1-28 and related mixtures.
45. Method according to claims 42-44 in which cells are those of higher organisms, including mammals and humans.
46. Method according to claims 42-44 in which cells are those of L929 cell line or human keratinocytes.

47. Method for a cosmetic treatment that uses at least one compound or product chosen according to claims 1-28 and relative mixtures.
48. Use of compounds or products according to claims 1-28 to modify MPS of eukaryotic cells.
49. Use according to claim 48 in which cells are those of higher organisms, including mammals and humans.
50. Use according to claim 48 in which cells are those of L929 cell line or human keratinocytes.
51. Use of compounds and products according to claims 1-28 to induce a heat shock response under stress condition such as , during heat shock, in eukaryotic organisms.
52. Use according to claim 51 in which cells are those of higher organisms, including mammals and humans.
53. Use according to claim 51 in which cells are those of L929 cell line or human keratinocytes.
54. Use of compounds and products according to claims 1-28 to protect eukaryotic organisms from stress.
55. Use according to claim 54 in which cells are those of higher organisms, including mammals and humans.
56. Use according to claim 54 in which cells are those of L929 cell line or human keratinocytes.
57. Use of compounds and products according to claims 1-28 to produce pharmacological agents for the treatment of pathological conditions due to an alteration of MPS of eukaryotic cells.
58. Use according to claim 57 in which cells are those of higher organisms, including mammals and humans.
59. Use according to claim 57 in which cells are those of L929 cell line or human keratinocytes.
60. Use according to claims 57-59 in which the alteration of MPS is due to at least one of the following stress conditions: oxidative stress, localized mechanic stress, osmotic stress, stress due to hypoxia ischemia, heat shock, UV radiations, by toxic compounds and free radicals.

61. Use according to claims 57-60 in which the pathological state is due to an alteration of MPS is in the following group: diabetes, vascular and cardiovascular diseases, coronary and cerebral diseases, allergies, immune and auto immune diseases, of viral or bacterial origin, tumors, skin diseases or of the mucosa, epithelial, renal, trauma, neurodegenerative diseases, dementia, Alzheimer, Parkinson, AIDS, epilepsy, physiological stress, ulcers, dermatitis, psoriasis burns.

62. Use of compounds and products according to claims 1-28 to produce pharmacological agents to treat and/or to prevent one of the following illness: chronic degenerative illness, cardiac cerebral ischemia, diabetes, vascular and cardiovascular diseases, coronary and cerebral diseases, allergies, immune and auto immune diseases, of viral or bacterial origin, tumors, skin diseases or of the mucosa, epithelial, renal, trauma, neurodegenerative diseases, dementia, Alzheimer, Parkinson, AIDS, epilepsy, physiological stress, ulcers, dermatitis, psoriasis burns.

63. Use of compounds and products according to claims 1-28 to produce cosmetics.

64. Method for a molecular assay to evaluate the activity of chemical compounds that modify MPS for use as pharmaceutical agents, dermatological and/or cosmetic products, such method comprising the following steps:

- Preparation of a vector containing a *reporter* gene coding for luciferase (or GFP, *green fluorescent protein*) under the control of a stress inducible *hsp70* promoter in mammalian or human cells;
- genetic transformation of mammalian cell lines with such vectors;
- treatment of the cell lines with the chemical compound of interest and subsequent exposure to stress;
- assay of the protein product (luciferase or determination of fluorescence of GFP) after exposure to stress;
- determination of anisotropy in the same cell lines do determine the changes in MPS.

65. Method according to claim 64 in which, rather than using a *reporter* gene heat shock gene transcription is determined by Northern blot directly measuring *hsp70* mRNA.

66. Method according to claim 64 in which, rather than using a *reporter* gene, heat shock gene transcription is determined by quantitative determination of *hsp70* mRNA.

67. Method according to claim 64 in which the cell lines are fibroblasts and keratinocytes.

68. Method according to claim 64 in which the stress is a heat shock for variable periods between 20 min to an hour or more.

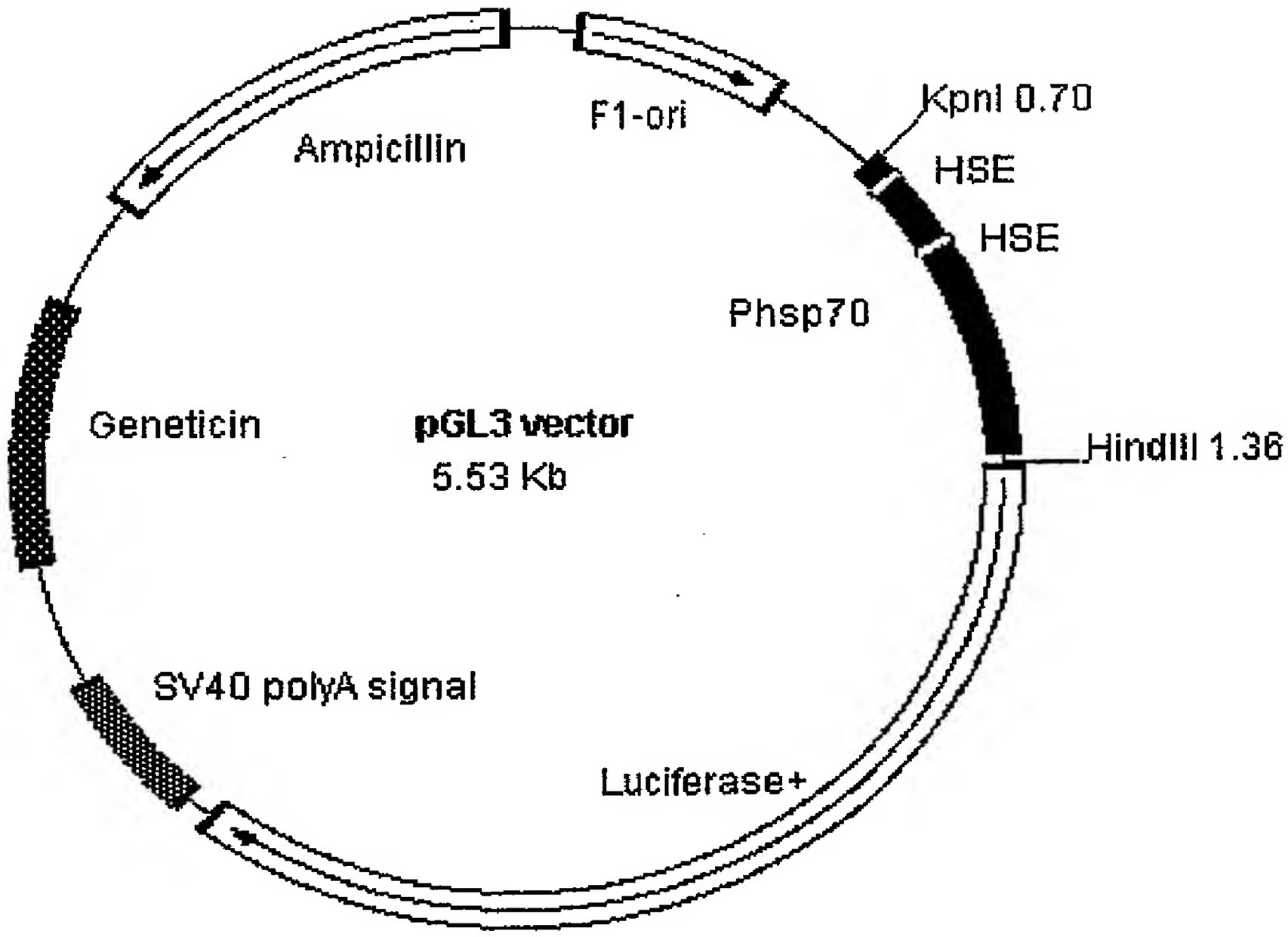


Fig. 1

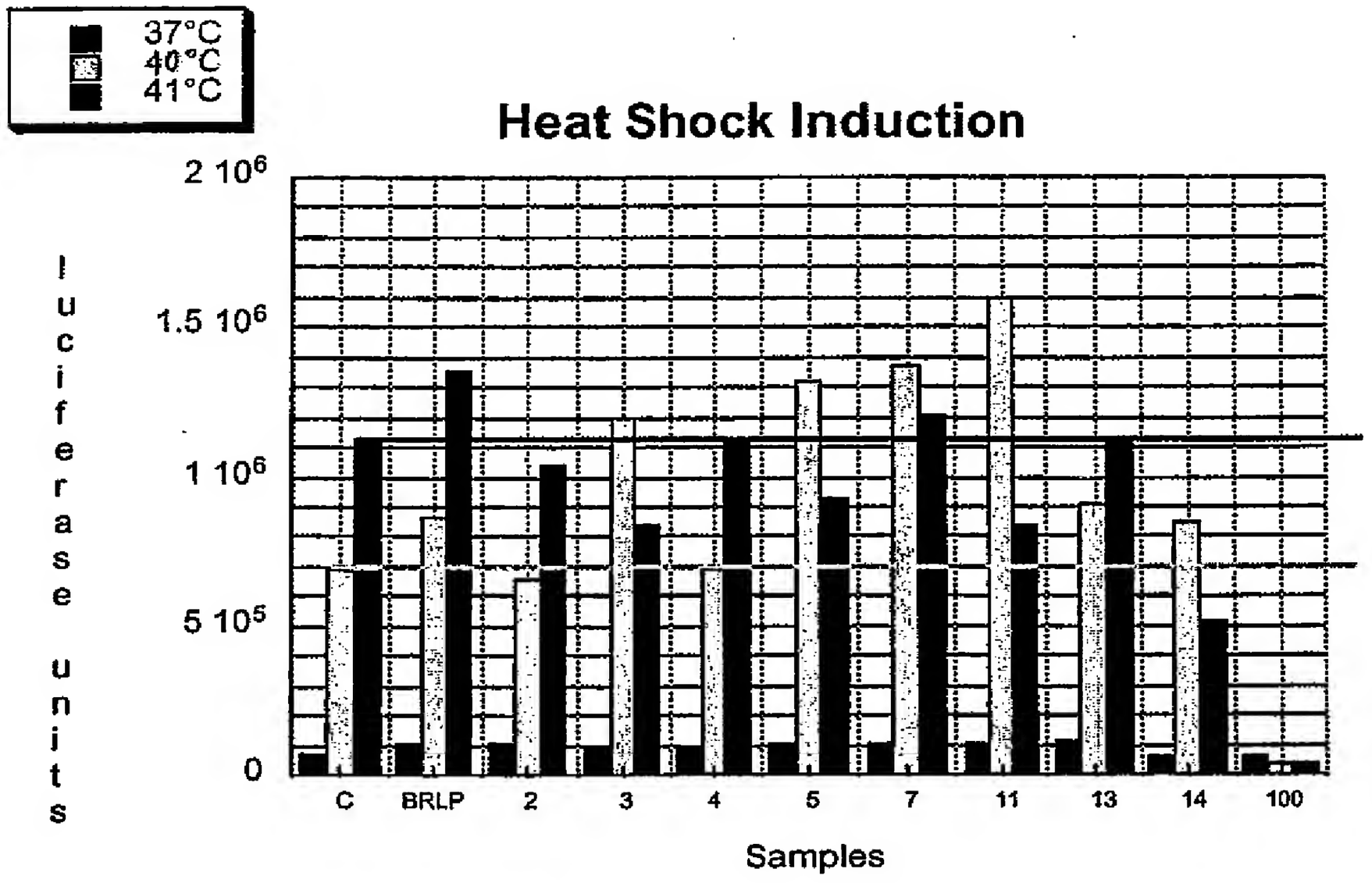


Fig. 2

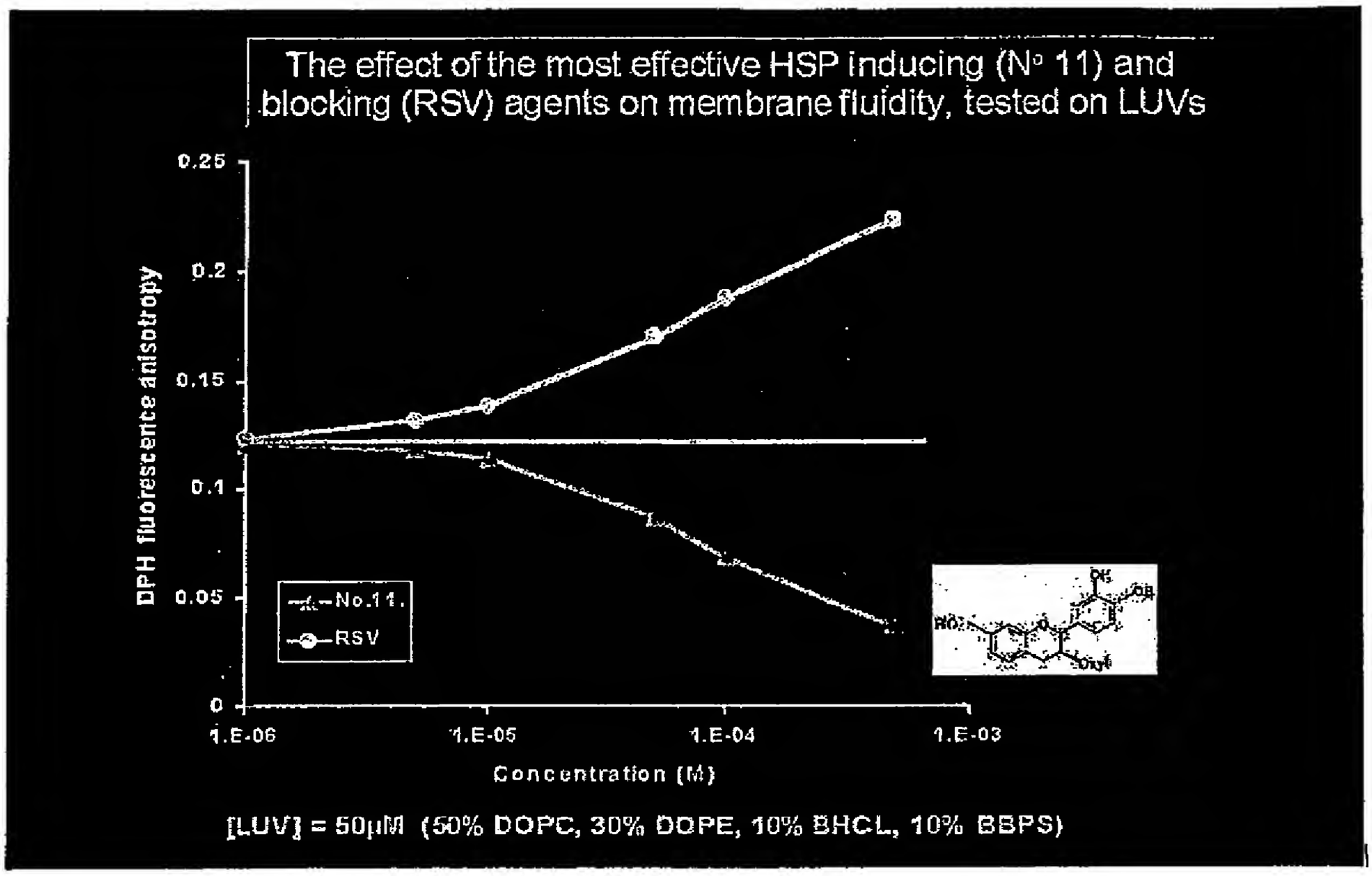


Fig. 3

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 April 2003 (17.04.2003)

PCT

(10) International Publication Number
WO 2003/031430 A3

(51) International Patent Classification[7]: C07D 311/62, 311/30, C07H 17/065, 17/07, A61P 17/00, A61K 31/352, G01N 33/50

(21) International Application Number: PCT/EP2002/011181

(22) International Filing Date: 4 October 2002 (04.10.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
RM2001A000600 4 October 2001 (04.10.2001) IT

(71) Applicant *(for all designated States except US)*: **BRANE TECH S.R.L.** [IT/IT]; Via Dora, 2, I-00198 Roma (IT).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **PORTA, Amalia** [IT/IT]; Via Giardini 1, I-88004 Crotone (IT).

(74) Agent: **GERVASI, Gemma**; Notarbartolo & Gervasi S.p.A., Corso di Porta Vittoria, 9, I-20122 Milan (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

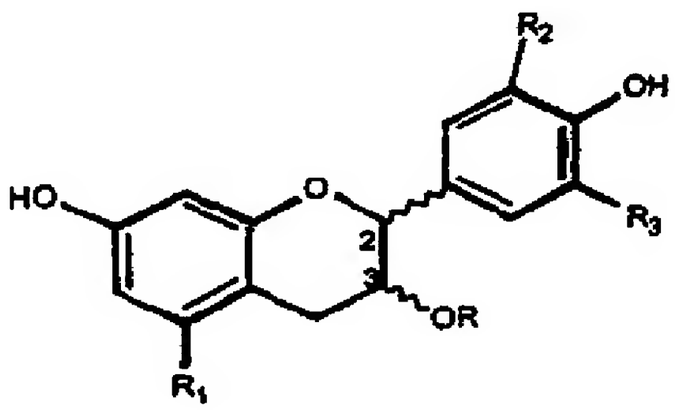
Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

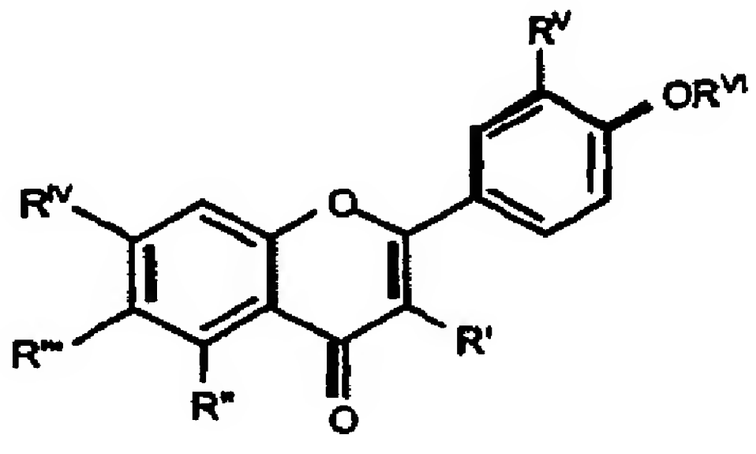
(88) Date of publication of the international search report:
8 April 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FLAVONOID COMPOUNDS AND THEIR PHARMACEUTICAL USES



(I)



(II)

(57) Abstract: The invention relates to flavonoids compounds of formula (I) and (II) capable of modifying the dynamic and/or physical state of biological membranes and to stimulate the endogenous synthesis of stress proteins in eukaryotic cells. Such compounds are molecules of plant origin or synthetic. The invention also describes a method to identify, purify and chemically synthesize such flavonoid compounds and test their efficacy through their capacity to stimulate the transcription of stress genes and as a consequence, to interact with biological membranes with alteration of their relative physical state. Such compounds and corresponding pharmaceutically acceptable derivatives and/or salts have applications in the areas of pharmaceuticals, more specifically in cosmetics and dermatology, for all those afections related to an alteration of the expression of stress genes.

WO 2003/031430 A3

INTERNATIONAL SEARCH REPORT

Interna Application No
PCT/EP 02/11181

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D311/62 C07D311/30 C07H17/065 C07H17/07 A61P17/00
A61K31/352 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C07D C07H A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GIDEON M.POLYA: "INHIBITION OF EUKARYOTE SIGNAL-REGULATED PROTEIN KINASES" PHYTOCHEMISTRY, vol. 35, no. 6, 1994, pages 1399-1405, XP008021995 GREAT-BRITAIN page 1399 - page 1404	1,5,25, 26,29, 61,62
X	I.DUARTE SILVA: "CHEMICAL FEATURES OF FLAVONOLS AFFECTING THEIR GENOTOXICITY" CHEMICO-BIOLOGICAL INTERACTIONS, vol. 124, no. 1, 2000, pages 29-51, XP001166889 page 29 - page 35	1,14, 20-22, 29,42, 61,62

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 January 2004	11. 02. 2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Griffith, G

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	TAKAHIKO ITO ET AL.: "PROTEIN SYNTHESIS INHIBITION BY FLAVONOIDS." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS., vol. 265, no. 2, 1999, pages 589-93, XP001166883 ACADEMIC PRESS INC. ORLANDO, FL., US ISSN: 0006-291X page 589 - page 593	1,10,11, 29,61,62
X	WO 99/62478 A (BEIERSDORF) 9 December 1999 (1999-12-09) the whole document	1,10-12, 22,25, 26,28, 61,62
X	MARCO SORIANI: "MODULATION OF THE UVA ACTIVATION OF HAEM OXYGENASE" FEBS LETTERS., vol. 439, no. 3, 1998, pages 253-257, XP004258666 ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM., NL ISSN: 0014-5793 page 253 - page 255	1,10-12, 21,22,60
X	GB 875 164 A (NAT. RESEARCH DEVELOPM.) 16 August 1961 (1961-08-16) page 1 - page 13; examples 5,10	1,16,21
X	DE 17 93 025 A (SORISA) 24 February 1972 (1972-02-24) page 1 - page 3; claims	1,16,29, 30,61,62
X	WO 92/13851 A (MANCUSO,S.) 20 August 1992 (1992-08-20) page 7; claims	1,14,28, 61,62
X	WO 97/27177 A (USA,DEPARTMENT OF HEALTH) 31 July 1997 (1997-07-31) claims 17,24; example 36; table 6	1,16,19, 29
X	WO 00/26207 A (EUROPHARMA) 11 May 2000 (2000-05-11) page 1 - page 7	1,16,19, 22,23
X	EP 0 052 086 A (BONOMELLI) 19 May 1982 (1982-05-19) the whole document	1,3,4, 57-61
X	US 5 646 178 A (E.B. WALKER) 8 July 1997 (1997-07-08) column 2; claims 1,4	1,2,14, 25,61,62

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/11021 A (ENDOLUMINAL THERAP.) 18 April 1996 (1996-04-18) page 0; claims	1,29, 43-45,60
X	CHEMICAL ABSTRACTS, vol. 127, no. 13, 1997, Columbus, Ohio, US; abstract no.: 181134f, page 1050 XP002255505 abstract & JP 09 176010 A (KUREHA) 8 July 1997 (1997-07-08)	1,25-27, 29,30, 43,61,62
X	CHEMICAL ABSTRACTS, vol. 117, no. 15, 1992, Columbus, Ohio, US; abstract no.: 144682f, HOSOKAWA,N.: "INHIBITION OF THE ACTIVATION OF HEAT SHOCK FACTOR IN VIVO A. IN VITRO BY FLAVONOIDS" page 220 column 1 XP002255506 abstract & MOL. CELL. BIOL., vol. 12, no. 8, 1992, pages 3490-8,	1,16,43
X	CHEMICAL ABSTRACTS, vol. 97, no. 1, 1982, Columbus, Ohio, US; abstract no.: 208015u, SUDHA,P.: "INTERACTION OF LUTEOLIN-7-O-GLUCOSIDE" page 61 XP002255507 abstract & AGROGYA(MANIPAL,INDIA), vol. 8, no. 2, 1982, pages 158-61, INDIA	1,3
X	CHEMICAL ABSTRACTS, vol. 131, no. 6, 1999, Columbus, Ohio, US; abstract no.: 78166b, page 1154 column 2 XP002255508 abstract & JP 11 180850 A (JAPAN LIFE CO.) 6 July 1999 (1999-07-06)	1,5,22, 23,25, 26,63
X	CHEMICAL ABSTRACTS, vol. 126, no. 33, 1997, Columbus, Ohio, US; abstract no.: 171838d, page 648 column 1 XP002255509 abstract	1,2,23, 25,26, 28,29, 62,63

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
	& JP 00 903089 A (MITSUI NORIN) 7 January 1997 (1997-01-07)	
X	CHEMICAL ABSTRACTS, vol. 119, no. 7, 1993, Columbus, Ohio, US; abstract no.: 198328e, MAKIMURA,M. ET AL.: "INHIBITORY EFFECT OF TEA CATECHINS ON COLLAGENASE" page 375 column 1 XP002255510 abstract & J. PERIODONTOL., vol. 64, no. 7, 1993, pages 630-6,	1,42-45, 48,49
X	EP 0 810 222 A (BERKEM) 3 December 1997 (1997-12-03) page 1 - page 4	25-28
P,X	WO 02/19965 A (BOEHRINGER) 14 March 2002 (2002-03-14) page 1; claims 1-26	1,24,43, 60-62
Y	SANDMAN KAREN E ET AL: "Rapid fluorescence-based reporter-gene assays to evaluate the cytotoxicity and antitumor drug potential of platinum complexes" CHEMISTRY AND BIOLOGY (LONDON), vol. 6, no. 8, August 1999 (1999-08), pages 541-551, XP002266449 & ISSN: 1074-5521 the whole document	64-68
Y	COOKSEY ROBERT C ET AL: "A rapid method for screening antimicrobial agents for activities against a strain of Mycobacterium tuberculosis expressing firefly luciferase" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 37, no. 6, 1993, pages 1348-1352, XP0000995193 & ISSN: 0066-4804 the whole document	64-68
Y	UCHINO SHIGEO ET AL: "Inducible expression of N-methyl-D-aspartate (NMDA) receptor channels from cloned cDNAs in CHO cells" MOLECULAR BRAIN RESEARCH, vol. 44, no. 1, 1997, pages 1-11, XP002266450 & ISSN: 0169-328X the whole document	64-68

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/11181

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9962478 A	09-12-1999	DE 19824727 A1	09-12-1999
		WO 9962478 A1	09-12-1999
		EP 1082100 A1	14-03-2001
		JP 2002516835 T	11-06-2002
GB 875164 A	16-08-1961	NONE	
DE 1793025 A	24-02-1972	DE 1793025 A1	24-02-1972
		FR 1578715 A	22-08-1969
		GB 1211311 A	04-11-1970
		US 3598840 A	10-08-1971
WO 9213851 A	20-08-1992	IT 1244647 B	08-08-1994
		AU 1269192 A	07-09-1992
		EP 0570475 A1	24-11-1993
		WO 9213851 A1	20-08-1992
		JP 7502009 T	02-03-1995
WO 9727177 A	31-07-1997	AU 709190 B2	26-08-1999
		AU 2246697 A	20-08-1997
		CA 2244774 A1	31-07-1997
		EP 0885192 A1	23-12-1998
		JP 2000516910 T	19-12-2000
		WO 9727177 A2	31-07-1997
		US 6066642 A	23-05-2000
WO 0026207 A	11-05-2000	IT FI980238 A1	02-05-2000
		AU 1043100 A	22-05-2000
		WO 0026207 A1	11-05-2000
		EP 1124816 A1	22-08-2001
		US 6656485 B1	02-12-2003
EP 0052086 A	19-05-1982	IT 1134205 B	13-08-1986
		AT 17733 T	15-02-1986
		DE 3173672 D1	13-03-1986
		EP 0052086 A1	19-05-1982
US 5646178 A	08-07-1997	US 5650432 A	22-07-1997
		US 5525341 A	11-06-1996
		AU 703158 B2	18-03-1999
		AU 5370096 A	16-10-1996
		BR 9607939 A	30-11-1999
		CA 2214464 A1	03-10-1996
		CN 1179104 A ,B	15-04-1998
		EP 0814825 A1	07-01-1998
		JP 11502849 T	09-03-1999
		NZ 513644 A	29-04-2003
		WO 9630033 A1	03-10-1996
WO 9611021 A	18-04-1996	US 5914345 A	22-06-1999
		AU 697201 B2	01-10-1998
		AU 4150296 A	02-05-1996
		CA 2202272 A1	18-04-1996
		EP 0785801 A1	30-07-1997
		JP 10509426 T	14-09-1998
		WO 9611021 A2	18-04-1996
		US 6071956 A	06-06-2000

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/11181

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 09176010 A	08-07-1997	NONE	
JP 11180850 A	06-07-1999	NONE	
JP 0903089 A	30-03-1978	JP 49130432 A	13-12-1974
		JP 52026249 B	13-07-1977
EP 0810222 A	03-12-1997	FR 2749303 A1	05-12-1997
		CA 2202297 A1	30-11-1997
		DE 69705159 D1	19-07-2001
		DE 69705159 T2	14-03-2002
		EP 0810222 A1	03-12-1997
		ES 2158460 T3	01-09-2001
		GR 3036569 T3	31-12-2001
		JP 10045611 A	17-02-1998
		PT 810222 T	28-09-2001
		US 5928646 A	27-07-1999
WO 0219965 A	14-03-2002	AU 9062901 A	22-03-2002
		WO 0219965 A2	14-03-2002